Cardiovascular abnormalities and impaired exercise performance in adolescents with Congenital Adrenal Hyperplasia

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Context: Patients with classic Congenital Adrenal Hyperplasia (CAH) are treated with lifelong glucocorticoids (GCS). Cardiovascular (CV) and metabolic effects of such therapy in adolescents have never been quantified.

Objective: To investigate left ventricular (LV) morphology, function and exercise performance in adolescents with CAH.

Design and Setting: cross-sectional and controlled study conducted at a tertiary referral centre.

Patients: Twenty patients with classic CAH (10 females) aged 13.6±2.5 years and 20 healthy controls comparable for sex and pubertal status **were enrolled in the study and compared to a group of 18 patients without CAH receiving a similar dose of GCS for Juvenile Idiopathic Arthritis (JIA).**

Main Outcomes Measures: Echocardiographic assessment and symptom-limited exercise testing were performed. Anthropometric, hormonal and biochemical parameters were also measured.

Results: Compared to healthy controls, patients with CAH exhibited an increased BMI ($p<0.001$), waist-to-height ratio ($p<0.001$), percentage of body fat ($p<0.001$) as well as higher insulin **concentrations** and HOMA index even after adjustment for BMI ($p=0.03$ and $p=0.05$, respectively).

Moreover, CAH patients exhibited an impaired exercise capacity as shown by reduced peak workload (99±27 *vs* 126±27 W, $p<0.01$) and higher systolic blood pressure response at peak (156±18 *vs* 132±11 mmHg, $p<0.01$; Δ=45±24 *vs* 22±10 mmHg, $p=0.05$) with respect to **healthy** controls.

CAH males displayed mild LV diastolic dysfunction as documented by significant prolongation of both isovolumic relaxation time (IRT) (118±18 vs 98±11ms, $p<0.05$) and mitral deceleration time (MDT) (138±25 *vs* 111±15 ms, $p<0.01$). No significant differences in CV function were found between CAH and JIA patients.

Conclusion: Adolescents with CAH exhibit impaired exercise performance and enhanced systolic blood pressure response during exercise. In our population, such abnormalities appear related to GCS therapy rather than CAH *per se*. CAH males, but no females, present mild LV diastolic dys-

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Abbreviations:

function that correlates with testosterone **concentrations** suggesting a sex hormone related difference.

Congenital Adrenal Hyperplasia (CAH) is a group of genetically transmitted enzymatic defects of glucocorticoid biosynthesis associated with insufficient cortisol production and accumulation of intermediate precursors (1). The 21-hydroxylase deficiency causes about 95% of cases (1). CAH is commonly divided into the severe classic and the milder nonclassic form. **All CAH phenotypes are associated with a salt-loss continuum.** Classic CAH is generally subdivided, depending on the extent of enzymatic impairment, into the salt-wasting form (SW), presenting with both cortisol and aldosterone deficiency, and the simple virilizing form (SV) characterized by an isolated cortisol deficiency. Both conditions are associated with androgen excess resulting in virilization of female external genitalia.

In the classic form steroid treatment is necessary to prevent adrenal crisis and suppress androgen excess. However, the therapeutic window is narrow, and supra-physiological doses of glucocorticoids (GCS) are often used to suppress androgen overproduction, especially in early infancy (2). As a result, CAH patients may develop iatrogenic Cushing's syndrome (1, 2), a condition that in adults and children is associated with insulin-resistance, hypertension and increased cardiovascular morbidity (3). **On the other hand, it is not even clear whether the negative cardio-metabolic effects are likely to be related to glucocorticoids excess or, conversely, to reduced androgen production, that could have a detrimental impact on the cardiovascular (CV) system** (4, 5).

Finally, low plasma epinephrine **concentrations** have been found in CAH, probably due to the lack of endogenous cortisol that normally promotes the development of adrenal medulla (1). Epinephrine deprivation is likely to affect exercise performance and may also have an additional detrimental effect on insulin-sensitivity (6).

In this intricate scenario, it is reasonable to expect a high cardiovascular risk profile in CAH patients, as suggested by the increasing number of studies showing a higher risk of obesity, insulin-resistance and high blood pressure (BP) in CAH children compared to the general population (7). However, the impact of these risk factors on heart structure and performance has never been systematically ascertained. Aim of the current study was to assess early CV risk factors, left ventricular (LV) morphology and function and exercise capacity **in adolescents with CAH, matched heathy controls and GC-treated non-CAH subjects with Juvenile Idiopathic Arthritis.**

Patients and Methods

Patients

Twenty CAH patients (10 females and 10 males), aged 13.6 ± 2.5years, were enrolled in the study. All patients were affected by classic CAH, with 15 having **the** SW (9 males) and 5 **the** SV form (1 male). Diagnosis was made on the basis of clinical evidence, elevated basal serum 17-OH-Progesterone (17OHP), elevated plasma renin in the SW forms, and confirmed by genetic tests in all subjects. Mean age at diagnosis was 1.4 ± 2.1 year. At study entry the mean duration of hydrocortisone therapy was 12.2 ± 3.3 years with a mean dose of 15.0 ± 3.9 mg/m^2/die, given three times daily. **The 50% of the total daily amount was administered in the morning, while the other two doses included 25% of the daily dose each. The three doses of hydrocortisone were given at 07:00 a.m., 3:00 p.m., 11:00 p.m., respectively.** Patients affected by SW CAH also received 9α-fludrocortisone at a mean dose of 54.8 ± 22.6 mcg/m^2/die. The adequacy of steroid therapy was monitored periodically in accordance with current guidelines (2). None of the patients used chronic treatments other than steroids. Known comorbidities such as liver failure, kidney failure, heart disease and other concomitant chronic diseases, as well as recurrent adrenal crisis in the 5 years before the study entry, were considered as exclusion criteria.

Twenty healthy adolescents, **statistically not different** for sex, pubertal status and physical activity were recruited as control group from those attending the outpatient clinic for a general pediatric control at "Federico II" University Hospital. Furthermore, in order to evaluate the influence of long-term glucocorticoid treatment on CV function, independent of CAH per se, we enrolled 18 (9 males) age- (13.4 ± 3.4 years) and BMI- SDS (**0.94 ± 0.88**) matched patients affected by Juvenile Idiopathic Arthritis (JIA) treated with **similar** doses of GCS (17.44 ± 9.1 mg/m^2/die) followed at Rheumatology facilities of the "Federico II" University. Written informed consent was obtained from the parents of all patients and controls. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and it was approved by the Ethics Committee of the Federico II University of Naples.

Study Protocol

At study entry both CAH patients and controls underwent a complete physical examination including measures of height, weight, waist and hip circumferences, heart rate. Body Mass Index (BMI) was calculated as Kg/m^2. BMI was normalized by age and sex and expressed as Standard deviation score (SDS) in accordance with Italian reference standards (8). Obesity was defined by a BMI SDS $>$ 2. Waist circumference was measured at the midpoint between the lower edge of the ribs in the mid-axillary line and the top of the iliac crest by the same clinician. Waist-to-height ratio (WtHR) was then calculated as an index of visceral adiposity. Pubertal status was assessed according to Tanner staging (9, 10). **Bone age was determined by using the TW2 method by two independent investigators** (11). Height per bone age was than calculated and expressed as SDS in accordance with Italian reference standards (8). Total body fat percentage was assessed by dual-energy X-ray absorptiometry (DEXA).

Systolic and diastolic blood pressures were measured in all subjects in the right arm with a standard sphygmomanometer by the same operator. Full blood count and coagulation status were obtained from all subjects. Evaluation of lipid profile was performed, measuring total and HDL cholesterol and triglycerides. LDL cholesterol was calculated by using the Friedewald formula. Fasting blood samples for measurement of insulin and glucose levels were obtained. Insulin-resistance was estimated using the HomeOstasiticModelAssessment (HOMA) method according to the formula: IR=insulin (mU/ml) x glucose (mmol/l)/22.5 (12). Coagulation state was assessed by measuring prothrombine time (PT), activate partial thromboplastine (aPTT), and fibrinogen levels. The adequacy of hormonal therapy at the time of the study was assessed by measuring ACTH, testosterone, 17-OHP, androstenedione (Δ4-A), DHEA-S, plasma renin and serum electrolytes. Inhibin B **concentrations** were assessed in male subjects to assess Sertoli cell function. To evaluate any influence of gender or enzymatic impairment degree on cardiovascular and metabolic features of CAH patients, males and females, as well as SW and SV patients, were separately assessed. In order to assess the effect of treatment on metabolic and CV parameters we calculated the mean hydrocortisone dosage in the 3 years before the study and we also designed an index of overtreatment (number of episodes of serum 17-OHP < 0.2 ng/ml during the last three years of follow-up). Those subjects who had an index of overtreatment ≥ 3 were considered as overtreated.

Echocardiography

An ultrasound system equipped with a 2.5 MHz multifrequency transducer (Aplio, Toshiba, Japan) was used for complete M-mode, two-dimensional, Doppler and Tissue Doppler Imaging (TDI) echocardiographic analyses. M-mode and two-dimensional recordings were made according to current guidelines (13). Measures of LV end-diastolic volume (EDV) and end-systolic volume (ESV) were measured by the modified Simpson's rule (13). Accordingly ejection fraction (EF) was calculated as follows: EF = (EDV-ESV)/ESV*100. LV mass was calculated according with America Society of Echocardiography-recomanded formula : LV mass = 0.8*{1.04[(LVIDd + PWTd + SWTd)^3-(LVIDd)^3]} + 0.6 g where PWTd and SWTd are posterior wall thickness at end diastole and septal wall thickness at ed, respectively (13). The following parameters of diastolic function were measured as the mean of three to five consecutive beats: diastolic transmitral peak velocities, E/A ratio, Isovolumic Relaxation Time (IRT), mitral deceleration time and pulmonary vein velocities. Quantitative diastolic data were derived from TDI analysis. Height^{2,7} was used for indexing LV cavity size. This method has showed to be more sensible in identifying overweight subjects with LV hypertrophy (14). Measurement of all parameters was performed off-line by one experienced investigator blind to the study protocol (M.A.).

Exercise Test

All subjects underwent an incremental symptom-limited exercise test on a bicycle ergometer following *Bruce protocol* (Yorba Linda, CA). We used this test for the evaluation of exercise capacity and BP levels at peak workload with 3-minutes step increments of 25 Watts. All patients were monitored by EKG for all the exercise duration. The exercise was stopped at the achievement of the target heart rate (defined as the 85% of 220 - chronological age in years) or when participant refused to continue the exercise test, despite verbal encouragement from the research staff. Maximal heart rate, SBP and DBP during the first minute of each step were recorded at the end of the test. All tests were closely physician-monitored and performed in the morning, after an overnight fast. The morning doses of HC and fludrocortisone were administered at the end of the test. All patients familiarized with the ergometer at least one week before the test, in order to avoid psychological stress.

Statistics

Statistical analysis was performed using SPSS (Chicago, IL, USA). Data are expressed as mean± Standard Deviation (SD). Variables were analyzed by Kolmogorov-Smirnov test and positively skewed variables were log-transformed for analysis. Independent t test for unpaired samples, was used to compare patients and controls. Since patients and controls were **statistically not different** for sex and pubertal status but not for BMI, an additional evaluation between groups was performed, using a linear regression model (ANCOVA) after adjustment for BMI. Bivariate correlations were calculated between hormonal and cardiovascular parameters. Significance was set at 5%.

Results

Table 1 describes the anthropometric, biochemical and hormonal characteristics of patients and **healthy** controls: males and females are reported separately. All subjects had entered puberty before the study entry. Seven females with CAH experienced menarche at a mean age of 11.5 years **statistically not different** with the control group. All patients who experienced menarche had regular menses. Bone age in the patients group was advanced by an average of two years (range 0.5–2.7 yrs), compared to chronological age. Compared to healthy controls, adolescents with CAH exhibited increased visceral adiposity, as suggested by higher values of BMI SDS (1.02 ± 0.92 vs -0.24 ± 1.5, $P = .0028$), waist circumference (83 ± 13 vs 72 ± 14 cm, $P < .01$), hip circumference (86 ± 9 vs 78 ± 12 cm, $P < .05$) and WtHR (0.55 ± 0.08 vs 0.47 ± 0.07 cm, $P < .001$), regardless of the sex. Moreover, DEXA analysis revealed higher values of total body fat (35 ± 8 vs 26 ± 8%, $P = .001$) in patients with CAH, with **similar** values of lean mass between the two groups (table 1).

Lipid profile, coagulation parameters, full blood count and fasting glycaemia were **similar** between patients and **healthy** controls.

Fasting insulin **concentrations** and HOMA index, although within normal values, were significantly higher in CAH patients, compared to **healthy** controls, even after adjustment for BMI ($P = .03$ and $P = .05$, respectively), thus suggesting a tendency toward reduced insulin sensitivity.

As expected, serum 17-OHP **concentrations** were significantly higher in CAH patients, compared to healthy

Table 1. Anthropometric, biochemical and hormonal characteristics of study population.

	ALL CASES		MALE		FEMALE	
	Controls (n = 20)	CAH (n = 20)	Controls (n = 10)	CAH (n = 10)	Controls (n = 10)	CAH (n = 10)
Age (years)	13.7 ± 2.4	13.6 ± 2.5	13.7 ± 1.7	14.0 ± 2.7	14.7 ± 2	13.0 ± 2.4
Height SDS	−0.52 ± 1.8	−0.49 ± 1.1	−0.57 ± 1.2	−0.25 ± 1.2	−0.47 ± 2.3	−0.74 ± 0.1
Height per bone age SDS	−1.23 ± 0.48	−1.29 ± 0.71	−1.03 ± 0.28	−1.02 ± 0.71	−1.44 ± 0.61	−1.56 ± 0.65
BMI SDS	−0.24 ± 1.5	1.02 ± 0.92**	0.33 ± 1	1.26 ± 0.93*	−0.8 ± 1.7	0.78 ± 0.91 *
Waist circumference (cm)	72 ± 14	83 ± 13**	70 ± 14	88 ± 14**	76 ± 14	77 ± 10
Hip circumference (cm)	78 ± 12	86 ± 9*	74 ± 11	91 ± 10**	86 ± 11	83 ± 6
Waist/Height ratio	0.47 ± 0.07	0.55 ± 0.08***	0.48 ± 0.08	0.53 ± 0.08**	0.47 ± 0.06	0.56 ± 0.08*
Total Body fat (%)	26 ± 8	35 ± 8**	25 ± 5.8	31 ± 8*	27 ± 9	38 ± 6 *
Total Body lean mass (%)	61.3 ± 21.8	62.3 ± 7.8	61 ± 21.9	65 ± 9	61.6 ± 21.8	59.6 ± 5.38
White blood cell count (x$10^3/\mu$L)	7.4 ± 2.5	8.1 ± 1.6	6.5 ± 1.8	7.7 ± 1.5	8.3 ± 2.7	8.6 ± 1.8
Red blood cell count (x$10^6/\mu$L)	4.8 ± 0.4	4.9 ± 0.3	5.1 ± 0.3	5.2 ± 0.3	4.4 ± 0.2	4.7 ± 0.5
Hemoglobin (g/dL)	13.7 ± 1.2	13.9 ± 0.9	14.5 ± 0.8	13.8 ± 1.4	13.3 ± 0.7	12.9 ± 0.8
Hematocrit (%)	39.5 ± 3.4	41.2 ± 3.2	41.1 ± 3.2	42.8 ± 3.1	38.1 ± 3.1	39.5 ± 2.6
Total cholesterol (mg/dL)	152 ± 27	155 ± 25	152 ± 28	154 ± 30	150 ± 28	157 ± 21
LDL cholesterol (mg/dL)	82 ± 24	87 ± 22	87 ± 24	89 ± 25	71 ± 20	85 ± 19
HDL cholesterol (mg/dL)	61 ± 14	56 ± 12	59 ± 18	53 ± 11	63 ± 13	60 ± 14
Triglycerides (mg/dL)	53 ± 30	55 ± 20	50 ± 24	60 ± 20	59 ± 40	50 ± 21
Glycemia (mg/dL)	75 ± 6	72 ± 6	77 ± 6	73 ± 6	71 ± 7	70 ± 6
Insulinemia (μIU/mL)	5.2 ± 2.0	12.0 ± 7.0****	5.4 ± 5.7	11.1 ± 7.2*	4.8 ± 3.6	12.6 ± 8.3**
HOMA index	1.0 ± 0,4	2.0 ± 1.3**	1.0 ± 1	2.0 ± 1.4*	1.0 ± 0.6	2.0 ± 1.3*
PT (sec)	12.3 ± 0.7	12 ± 0.7	12.2 ± 0.5	11.7 ± 0.6	12.5 ± 0.5	12.3 ± 0.8
APTT (sec)	33.3 ± 3.6	33.6 ± 3.4	35.7 ± 2.4	33.2 ± 3.6	31.2 ± 3.1	34.0 ± 3.2
Fibrinogen (mg/dL)	301.9 ± 70.7	291.4 ± 60.1	310.4 ± 97.5	306.6 ± 50.4	294.3 ± 37.8	276.2 ± 67.6
ACTH (pg/ml)	29.1 ± 11	26.3 ± 23	27.2 ± 10.1	32.2 ± 27.4	31.2 ± 12.5	20.3 ± 16.9
17-OH Progesterone (ng/mL)	0.6 ± 0.3	12 ± 15**	0.92 ± 0.12	16.3 ± 20.5*	0.6 ± 0.4	7.9 ± 6.2**
DHEA-S (μg/dL)	45.3 ± 23.4	52.5 ± 64.4	44.4 ± 28.8	40.3 ± 28.7	46.2 ± 18.2	64.7 ± 87.1
Androstenedione (ng/dL)	-	-	70.7 ± 22.2	165 ± 162	87.5 ± 29.3	160 ± 118
Testosterone (ng/dL)	-	-	446.3 ± 257.5	226.7 ± 168.5*	27.4 ± 7.7	31.5 ± 8.8
Plasma renin (pg/mL)	-	26.3 ± 22.6	-	28.8 ± 27.7	-	23.8 ± 17.2
Hydrocortisone dose (mg/m2/day)	-	15.0 ± 3.9	-	16.6 ± 2.6	-	13.5 ± 4.6
9α-fludrocortisone dose (mcg/m2/day)	-	54.8 ± 22.6	-	60.9 ± 26.5	-	46.9 ± 14.7

Data expressed as mean±SD; ns, not significant.

CAH: Congenital Adrenal Hyperplasia; BMI: Body Mass Index; HOMA: HomeOstasis Model Assessment.

*$P < 0.05$ *vs.* controls; **$P < 0.01$ *vs.* controls; ***$P < 0.001$ *vs.* controls; ****$P < 0.0001$ *vs.* controls

controls. Despite the higher 17-OHP **concentrations**, CAH males showed lower testosterone **concentrations** when compared to male **healthy** controls ($P < .05$) (table 1). To assess Sertoli cell function we next measured inhibin B serum **concentrations** in male subjects. We did not found any difference between male CAH and healthy controls (100.0 ± 8.1 vs 96.3 ± 5.0 pg/ml, respectively; p: 0.25-data not shown in Table 1).

Table 2 shows CV parameters and exercise capacity in both CAH patients and **healthy** controls. No differences in resting systolic and diastolic BP as well as heart rate were observed between patients and **healthy** controls. Echocardiographic data showed that cavity size was **statistically not different** between groups, regardless of the indexing method used (Body surface area or height^{2,7}). CAH males displayed a significant prolongation of both isovolumic relaxation time (IRT) and mitral deceleration time (MDT), even after adjustment for BMI ($P = .001$ for both parameters), consistent with mild diastolic dysfunction. Furthermore, when considering separately SW and SV forms, they both showed the same pattern of CV impairment (Table 3). No differences in diastolic function and exercise capacity were observed between overtreated and normo-/undertreated patients (Table 3).

Interestingly, a significant negative correlation was found between serum testosterone **concentrations** and both IRT (r= −0.864, $P = .02$) and MDT (r= −0.764, $P = .001$), as well as between testosterone **concentrations** and mean hydrocortisone dosage (r= −0,7148, $P = 0,02$).

Both male and female CAH shared a pattern of impaired exercise capacity as documented by the reduced peak workload (figure 1). Moreover, CAH patients showed an exaggerated SBP at peak of exertion, regardless of the sex (Table 2). Differences in peak workload and SBP at peak of exertion remained all significant even after adjustment for BMI (adjusted $P = .03$ and 0.0001, respectively).

Bivariate analysis showed a significant positive correlation between HOMA index and both SBP (r = 0.65, $P = .001$), and ΔSBP (r = 0.55 $P < .05$), thus suggesting a pivotal role of insulin in BP control.

Patients with JIA, treated with doses of GCS similar to those employed in CAH, shared a similar CV phenotype. In fact, compared to healthy controls, patients with JAI displayed a mild diastolic dysfunction as well as an impaired exercise capacity (Table 4).

Discussion

The results of the present study show that our small cohort of adolescents with CAH exhibits mild LV diastolic dys-

Table 2. Cardiovascular status and exercise capacity in controls and Congenital Adrenal Hyperplasia (CAH) patients

	ALL CASES		MALE		FEMALE	
	Controls	CAH	Controls	CAH	Controls	CAH
	(*n* = 20)	(*n* = 20)	(*n* = 10)	(*n* = 10)	(*n* = 10)	(*n* = 10)
LV EDV index (ml/m^{2,7}-height)	27 ± 8	27 ± 9	27 ± 8	23 ± 11	26 ± 9	31 ± 3
LV ESV index (ml/m^{2,7}-height)	10 ± 3	11 ± 4	11 ± 3	10 ± 4	9 ± 2	12 ± 3
LA diameter (mm)	27.0 ± 4.3	29.5 ± 4.7	27.2 ± 4.4	31.1 ± 5.9	26.8 ± 4.4	28.0 ± 2.6
LA volume index (ml/m^{2,7}-height)	13.8 ± 3.4	15.2 ± 3.7	12.6 ± 3.3	14.5 ± 4.9	15.0 ± 3.1	15.5 ± 1.2
Relative Wall Thickness	0.37 ± 0.04	0.36 ± 0.04	0.36 ± 0.05	0.37 ± 0.04	0.38 ± 0.03	0.35 ± 0.03
LV-mass index (g/m^{2,7}-height)	33 ± 10	35 ± 12	30 ± 8	31 ± 16	35 ± 12	40 ± 7
Ejection Fraction (%)	60 ± 9	60 ± 6	58 ± 7	59 ± 5	62 ± 11	62 ± 7
MDT (ms)	116 ± 18	138 ± 22***	111 ± 15	138 ± 25**	122 ± 20	137 ± 20
IRT (ms)	101 ± 13	116 ± 15*	98 ± 11	118 ± 18**	104 ± 14	115 ± 10
E/A	2.3 ± 0.4	2.3 ± 0.7	2.3 ± 0.3	2.4 ± 0.7	2.3 ± 0.4	2.3 ± 0.8
E'/A'	3.0 ± 0.8	3.3 ± 0.9	3.2 ± 0.9	3.6 ± 1.2	2.7 ± 0.4	3.0 ± 0.5
E/E'	7.6 ± 1,4	7.4 ± 1.8	8.1 ± 1.4	7.4 ± 2.3	7.2 ± 1.2	7.4 ± 1.4
adur-A dur (ms)	14.6 ± 0.2	14.8 ± 0.4	14.2 ± 0.7	14.7 ± 0.5	14.9 ± 0.8	15.0 ± 0.3
PVa (m/sec)	0.25 ± 0.03	0.25 ± 0.01	0.25 ± 0.02	0.25 ± 0.02	0.24 ± 0.03	0.25 ± 0.04
Heart Rate (bpm)	92 ± 14	95 ± 19	96 ± 18	107 ± 15	87 ± 11	84 ± 14
Resting SBP (mmHg)	110 ± 7	111 ± 11	111 ± 7	110 ± 12	110 ± 5	111 ± 11
Resting DBP (mmHg)	67 ± 11	72 ± 7	68 ± 11	75 ± 6	66 ± 10	69 ± 7
Peak Workload (W)	126 ± 26	99 ± 27**	135 ± 26	102 ± 18**	117 ± 28	87 ± 24**
Peak Heart Rate (bpm)	198 ± 10	197 ± 9	196 ± 13	198 ± 12	200 ± 3	196 ± 12
Peak SBP (mmHg)	132 ± 11	156 ± 18**	133 ± 10	152 ± 17*	131 ± 12	159 ± 20**
Peak DBP (mmHg)	70 ± 10	76 ± 8	70 ± 12	77 ± 5	70 ± 7	75 ± 11
Δ SBP (mmHg)	22 ± 10	45 ± 24*	23 ± 12	46 ± 26*	22 ± 12	47 ± 24*
Δ DBP (mmHg)	3.0 ± 9	3.7 ± 10	1.5 ± 11	2.0 ± 7	4.5 ± 7	5.5 ± 13

Data expressed as mean ± SD; ns, not significant.

CAH: Congenital Adrenal Hyperplasia; LV: Left Ventricular; EDV: End-diastolic volume; ESV: End-systolic volume; LA: Left Atrial; MDT: Mitral Deceleration Time; IRT: Isovolumic Relaxation Time

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure;

Δ SBP (mmHg): difference between Resting SBP and peak SBP; Δ DBP (mmHg): difference between Resting DBP and peak DBP.

*$P < 0.5$ *vs.* controls; **$P < 0.01$ *vs.* controls; ***$P < 0.001$ *vs.* controls; ****$P < 0.0001$

function, impaired exercise performance and enhanced systolic BP response during exercise, compared to matched **healthy** controls. Furthermore, in our cohort both CAH males and females exhibit a cluster of metabolic alterations such as increased visceral adiposity and a tendency toward impaired insulin sensitivity, that may place them at an increased risk of CV diseases in adulthood, in agreement with recent studies [5]. Finally, such CV abnormalities appear related to GCS therapy rather than CAH per se.

To date, data on cardiovascular function in patients with CAH are scanty, however, impaired cardiac function has been reported in subject with hypercortisolism due to endogenous and exogenous Cushing's Syndrome (15), as well as in several others chronic endocrinopathies (16–20).

To our knowledge this is the first study evaluating cardiac function and exercise capacity in adolescents with CAH.

In a recent study, *Ubertini et al* (21) reported a normal LV mass in 20 young CAH patients. The study, however, did not provide data on LV cavity size, systolic and diastolic function. Another limit of the study was the absence of a control group. Consistently with *Ubertini* (21) we observed a normal LV morphology; moreover, for the first time, we documented the presence of a mild diastolic dysfunction, that was a distinctive feature of male CAH.

An abnormal relaxation pattern without elevated resting diastolic filling may be related to both impaired insulin-sensitivity (22) and obesity (23) through different mechanisms such as impaired insulin-signaling, glyco- and lipo-toxicity, increased cytokine activity and interstitial deposition of triacylglicerol and AGEs (24). In our patients, insulin and HOMA values, despite higher than **healthy** controls, were still within the normal range, thus their role in influencing diastolic function is questionable. Conversely, in our patients diastolic dysfunction was significantly associated with testosterone **concentrations**. Whether androgen deficiency plays a role in CV system development and function remains unknown, although recent studies on young adults with Klinefelter's syndrome point toward a possible association (18). In adults, chronic exposure to reduced androgens carry detrimental effects on metabolic profile and cardiovascular status (4, 5) and large epidemiological studies showed that low serum testosterone is an excellent biomarker of cardiovascular mortality (25). In fact, testosterone **concentrations** may exert a protective effect on the progression of atherosclerosis through both direct and mediated mechanisms (4, 5).

Our findings suggest that exposure to suboptimal testosterone levels during pubertal development, may have exerted detrimental effects on cardiovascular performance of males affected by CAH. In our patients, testosterone **concentrations** were negatively associated with

Table 3. Subgroup Analysis of diastolic function and exercise capacity in 1) Salt-wasting (SW) form patients *vs.* age-matched controls 2) Simply virilizing (SV) form patients *vs.* age-matched controls 3) Overtreated patients *vs.* normo-/undertreated patients.

	Age-matched controls	SW form	Age-matched controls	SV form	Overtreated CAH	Normo-/Under-treated
	(*n* = 15)	(*n* = 15)	(*n* = 5)	(*n* = 5)	(*n* = 6)	(*n* = 14)
MDT (ms)	111.8 ± 16.9	137.6 ± 23.8**	126.9 ± 18.2	138.6 ± 18.4	146.3 ± 26.0	134.2 ± 20.2
IRT (ms)	100.9 ± 13.1	114.6 ± 13.4**	101.4 ± 14.2	121.8 ± 18.4*	120.0 ± 19.7	114.9 ± 12.4
E/A	2.3 ± 0.4	2.3 ± 0.8	2.2 ± 0.9	2.3 ± 0.2	2.7 ± 0.9	2.1 ± 0.4
E′/A′	3.0 ± 0.8	3.3 ± 1.0	2.8 ± 0.7	3.2 ± 0.5	3.7 ± 1.4	3.2 ± 0.7
E/E′	7.8 ± 1.5	7.3 ± 2.1	7.3 ± 0.95	7.8 ± 0.8	6.6 ± 1.8	7.5 ± 1.8
Peak Workload (W)	126.0 ± 27.5	95.0 ± 25.5***	125.0 ± 25.0	95.0 ± 20.9*	87.5 ± 20.9	98.2 ± 22.9
Peak Heart Rate (bpm)	196.7 ± 10.7	197.1 ± 12.8	202.4 ± 6.2	195.4 ± 9.4	203.2 ± 8.9	193.9 ± 12.2
Peak SBP (mmHg)	133.0 ± 11.0	159.7 ± 19.9****	131.0 ± 12.5	143.0 ± 4.4*	156.6 ± 23.2	155.0 ± 17.4
Peak DBP (mmHg)	70.3 ± 10.6	75.0 ± 7.8	68.0 ± 7.5	78.0 ± 10.9	73.3 ± 8.2	76.8 ± 8.7
Δ SBP (mmHg)	24.4 ± 11.6	43.3 ± 27.0***	10.0 ± 12.5	30.0 ± 3.5*	45.0 ± 32.2	44.3 ± 22.3
Δ DBP (mmHg)	3.3 ± 9.75	3.6 ± 9.1	2.0 ± 5.7	4.0 ± 3.9	6.6 ± 12.1	2.5 ± 12.5

Patients were considered "overtreated" if they had ≥3 episodes of serum 17-OHP <0.2 ng/ml during the last three years of follow-up.

*$P < 0.5$ *vs.* controls; **$P < 0.01$ *vs.* controls; ***$P < 0.001$ *vs.* controls; ****$P < 0.0001$

SW: Salt-wasting form; SV: Simply-virilizing form; MDT: Mitral Deceleration Time; IRT: Isovolumic Relaxation Time

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure;

Δ SBP (mmHg): difference between Resting SBP and peak SBP; Δ DBP (mmHg): difference between Resting DBP and peak DBP.

mean hydrocortisone dosage in the 3 years before the study, thus suggesting that high hydrocortisone dosage during puberty, although still within the recommended range, may suppress testosterone production, thus increasing the risk of early CV abnormalities (26).

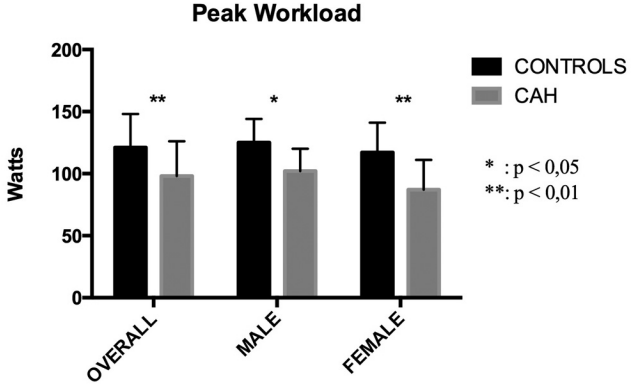

Figure 1.

Both males and females CAH shared a common pattern of exercise impairment and exaggerated BP response to exercise. Exercise capacity in CAH adolescents has been investigated both in short-term high intensity (6) and in long-term moderate intensity exercise (37). *Weise et al* (6) performed a maximal incremental exercise test to determine maximal aerobic capacity in nine CAH adolescents while *Green-Golan and coworkers* (27) investigated the cardiovascular response to prolonged moderate-intensity exercise in six adolescents with classic CAH. In contrast with our results, both studies did not find any difference in exercise capacity between CAH patients and **healthy** controls. The small sample size of these two studies as well as differences in the study population, (both studies evalu-

Table 4. Cardiovascular status and exercise capacity in Juvenile Idiopathic Arthritis (JIA) patients

	JIA Patients	*p value vs. Controls*	*p value vs. CAH*
	(*n* = 18)		
Age (years)	13.4 ± 3.4	ns	ns
BMI (kg/m^2)	**0.94 ± 0.88**	**0.01**	**ns**
Hydrocortisone dose (mg/m2/day)	17.44 ± 9.1	-	ns
LV EDV index (ml/m^{2,7}-height)	31 ± 5	ns	ns
LV ESV index (ml/m^{2,7}-height)	11 ± 3	ns	ns
LA diameter (mm)	29.5 ± 4.9	ns	ns
LA volume index (ml/m^{2,7}-height)	16.1 ± 5.6	ns	ns
Relative Wall Thickness	0.37 ± 0.03	ns	ns
LV-mass index (g/m^{2,7}-height)	33 ± 8	ns	ns
Ejection Fraction (%)	62 ± 5	ns	ns
MDT (ms)	155 ± 33	0.001	ns
IRT (ms)	117 ± 23	0.01	ns
E/A	2.1 ± 0.5	ns	ns
E'/A'	3.3 ± 0.9	ns	ns
E/E'	7.7 ± 2.5	ns	ns
adur-A dur (ms)	14.4 ± 0.4	ns	ns
PVa (m/sec)	0.24 ± 0.02	ns	ns
Heart Rate (bpm)	94 ± 16	ns	ns
Resting SBP (mmHg)	108 ± 14	ns	ns
Resting DBP (mmHg)	69 ± 8	ns	ns
Peak Workload (W)	86 ± 21	0.0001	ns
Peak Heart Rate (bpm)	197 ± 7	ns	ns
Peak SBP (mmHg)	154 ± 16	0.0001	ns
Peak DBP (mmHg)	72 ± 11	ns	ns
Δ SBP (mmHg)	47 ± 19	0.0001	ns
Δ DBP (mmHg)	2.7 ± 12.0	ns	ns

ns: non significant

JIA: Juvenile Idiopathic Arthritis; CAH: Congenital Adrenal Hyperplasia;MDT: Mitral Deceleration Time; IRT: Isovolumic Relaxation Time

SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure;

Δ SBP (mmHg): difference between Resting SBP and peak SBP; Δ DBP (mmHg): difference between Resting DBP and peak DBP

ated only nonobese CAH subjects), may account for the different results in exercise performance. In our study, however, differences in peak workload and SBP at peak of exertion remained all significantly higher than **healthy** controls even after adjustment for BMI. Moreover, a positive correlation was found between both peak SBP and ΔSBP and HOMA index. The relationship between insulin-resistance and hypertension is well-known (1, 28), however, despite higher insulin **concentrations** and HOMA index than **healthy** controls, our patients were not overtly insulin-resistant. Therefore, the hypothesis can be put forward that other factors, such as the detrimental effect of GCS on muscle performance (29) and the impaired release of epinephrine from the adrenomedullary of CAH patients secondary to an altered medulla organogenesis (1) may played a key role in determining such alterations.

Blood pressure control in CAH has been investigated by several independent groups, with some studies reporting normal resting (21, 30, 31) and 24h BP profile (32) and others reporting either diurnal (33) or both diurnal and nocturnal SBP significantly elevated (34) and positively related to increased BMI. In the study by *Ubertini et al* (21) systolic BP in response to exercise were substantially normal in CAH patients with good hormonal balance (35). However, the wide age span (5.1–17.49 years), and the lack of a control group might be responsible for the different results. In agreement with *Ubertini et al* (21), we found normal resting BP with a slight trend in higher DBP in CAH group (P = .09).

Overall these findings suggest that resting BP may be unaffected by CAH, while BP control during normal daily activities, as well as in response to exercise, may be impaired in CAH as a result of the metabolic disarray.

A similar pattern of cardiac abnormalities as well as of impaired exercise capacity was observed in the group of patients affected by JAI treated with **similar** doses of GCS, thus suggesting that CV impairment was not related to CAH per se but to a detrimental role of long-term GCS treatment.

Despite GCS administration has dramatically changed live expectancy of CAH patients, supra-physiological doses of GCS are often necessary to address several clinical problems (32). First, intrauterine glucocorticoid deficiency can lead to a condition of impaired postnatal pituitary sensitivity to feedback inhibition (36). Second, exogenous administration of GCS and mineralocorticoids cannot replicate the close temporal pattern of physiological cortisol and aldosterone pulses (7) so that physicians are tempted to overtreat in order to avoid crisis.

High CGS doses may, in turn, suppress testosterone production, as observed in our male with CAH, thus in-

creasing the risk of early CV abnormalities. **Although the aim of our study was not to provide any pathophysiological links between GCS replacement and CAH, our data suggest that CV abnormalities found in our study population are likely related to GCS administration. Indeed, a cohort of patients affected by JIA that were statistically comparable to our CAH sample for age, BMI and GCS dose, showed a similar pattern of CV abnormalities.**

Moreover, even though the evaluation of the effects of glucocorticoid therapy on CV status in the general prepubertal population was not the main aim of our study, this may represent a stimulus for future studies aimed at evaluating dose dependency, permanence of glucocorticoid therapy in children. Recent studies on Addison disease, the most common acquired adrenal failure (37), revealed an increased risk for cardiovascular death in patients of both sexes likely related to high doses of GCS (38). Because CAH patients are treated with even higher doses of GCS than Addison's patients, further longitudinal investigations from large international registries are necessary to assess such a risk in CAH patients.

In conclusion our data suggest, for the first time, that adolescents with CAH are at risk for subclinical cardiovascular abnormalities, such as diastolic dysfunction (39) and enhanced exercise BP (40). **A careful anthropometric and metabolic monitoring, especially during puberty, may be helpful in this clinical setting in order to implement early life-style modification and to prevent the development of visceral adiposity and insulin resistance related to glucocorticoid therapy, which in turn may led to CV abnormalities.** Moreover, CAH patients may exhibit increased abdominal adiposity, a trend toward insulin-resistance, and a low testosterone exposure in males, as a possible effect of overtreatment, that may play an additional detrimental role on CV performance.

Further studies on larger cohorts are necessary to better clarify the mechanisms leading to metabolic and cardiovascular abnormalities, in the meanwhile, additional effort to avoid the complete suppression of androgens **concentrations**, especially in males, should represent a therapeutic goal for improving management of peri-pubertal patients with CAH.

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References

1. **Merke DP, Bornstein SR.** Congenital adrenal hyperplasia. *Lancet*. 2005;365:2125–2136.
2. **Speiser PW, Azziz R, Baskin LS, Ghizzoni L, Hensle TW, Merke DP, Meyer-Bahlburg HF, Miller WL, Montori VM, Oberfield SE, Ritzen M, White PC.** Congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2010;95:4133–4160.
3. **Tritos NA, Biller BM, Swearingen B.** Management of Cushing disease. *Nat Rev Endocrinol*. 2011;7:279–289.
4. **Isidori AM, Greco EA, Aversa A.** Androgen deficiency and hormone-replacement therapy. *BJU Int*. 2005;96:212–216.
5. **Rosano GM, Sheiban I, Massaro R, Pagnotta P, Marazzi G, Vitale C, Mercuro G, Volterrani M, Aversa A, Fini M.** Low testosterone levels are associated with coronary artery disease in male patients with angina. *Int J Impot Res*. 2007;19:176–182.
6. **Weise M, Mehlinger SL, Drinkard B, Rawson E, Charmandari E, Hiroi M, Eisenhofer G, Yanovski JA, Chrousos GP, Merke DP.** Patients with classic congenital adrenal hyperplasia have decreased epinephrine reserve and defective glucose elevation in response to high-intensity exercise. *J Clin Endocrinol Metab*. 2004;89:591–597.
7. **Mooij CF, Kroese JM, Claahsen-van der Grinten HL, Tack CJ, Hermus AR.** Unfavourable trends in cardiovascular and metabolic risk in paediatric and adult patients with congenital adrenal hyperplasia? *Clin Endocrinol (Oxf)*. 2010;73:137–146.
8. **Cacciari E, Milani S, Balsamo A, Spada E, Bona G, Cavallo L, Cerutti F, Gargantini L, Greggio N, Tonini G, Cicognani A.** Italian cross-sectional growth charts for height, weight and BMI (2 to 20 yr). *J Endocrinol Invest*. 2006;29:581–593.
9. **Marshall WA, Tanner JM.** Variations in the pattern of pubertal changes in boys. *Arch Dis Child*. 1970;45:13–23.
10. **Marshall WA, Tanner JM.** Variations in pattern of pubertal changes in girls. *Arch Dis Child*. 1969;44:291–303.
11. **Tanner JM WR, Cameron N, Marshall WA, Healy MJR GH.** *Assessment of skeletal maturity and prediction of adult height, 2nd ed.*; 1983.
12. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
13. **Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MS, Stewart WJ.** Chamber Quantification Writing G, American Society of Echocardiography's G, Standards C, European Association of E. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. *J Am Soc Echocardiogr*. 2005;18:1440–1463.
14. **Cuspidi C, Giudici V, Negri F, Meani S, Sala C, Zanchetti A, Mancia G.** Improving cardiovascular risk stratification in essential hypertensive patients by indexing left ventricular mass to height(2.7). *J Hypertens*. 2009;27:2465–2471.
15. **De Leo M, Pivonello R, Auriemma RS, Cozzolino A, Vitale P, Simeoli C, De Martino MC, Lombardi G, Colao A.** Cardiovascular disease in Cushing's syndrome: heart versus vasculature. *Neuroendocrinology*. 2010;92 Suppl 1:50–54.
16. **Salerno M, Oliviero U, Lettiero T, Guardasole V, Mattiacci DM, Saldamarco L, Capalbo D, Lucariello A, Sacca L, Cittadini A.** Long-term cardiovascular effects of levothyroxine therapy in young adults

with congenital hypothyroidism. *J Clin Endocrinol Metab*. 2008; 93:2486–2491.
17. **Fazio S, Biondi B, Carella C, Sabatini D, Cittadini A, Panza N, Lombardi G, Sacca L.** Diastolic dysfunction in patients on thyroid-stimulating hormone suppressive therapy with levothyroxine: beneficial effect of beta-blockade. *J Clin Endocrinol Metab*. 1995;80: 2222–2226.
18. **Pasquali D, Arcopinto M, Renzullo A, Rotondi M, Accardo G, Salzano A, Esposito D, Saldamarco L, Isidori AM, Marra AM, Ruvolo A, Napoli R, Bossone E, Lenzi A, Baliga RR, Sacca L, Cittadini A.** Cardiovascular abnormalities in Klinefelter Syndrome. *Int J Cardiol*. 2012.
19. **Capalbo D, Lo Vecchio A, Farina V, Spinelli L, Palladino A, Tiano C, Lettiero T, Lombardi G, Colao A, Salerno M.** Subtle alterations of cardiac performance in children with growth hormone deficiency: results of a two-year prospective, case-control study. *J Clin Endocrinol Metab*. 2009;94:3347–3355.
20. **Salerno M, Esposito V, Farina V, Radetti G, Umbaldo A, Capalbo D, Spinelli L, Muzzica S, Lombardi G, Colao A.** Improvement of cardiac performance and cardiovascular risk factors in children with GH deficiency after two years of GH replacement therapy: an observational, open, prospective, case-control study. *J Clin Endocrinol Metab*. 2006;91:1288–1295.
21. **Ubertini G, Bizzarri C, Grossi A, Gimigliano F, Rava L, Fintini D, Cappa M.** Blood Pressure and Left Ventricular Characteristics in Young Patients with Classical Congenital Adrenal Hyperplasia due to 21-Hydroxylase Deficiency. *Int J Pediatr Endocrinol*. 2009;2009: 383610.
22. **von Bibra H, St John Sutton M.** Diastolic dysfunction in diabetes and the metabolic syndrome: promising potential for diagnosis and prognosis. *Diabetologia*. 2010;53:1033–1045.
23. **Peterson LR, Waggoner AD, Schechtman KB, Meyer T, Gropler RJ, Barzilai B, Davila-Roman VG.** Alterations in left ventricular structure and function in young healthy obese women: assessment by echocardiography and tissue Doppler imaging. *J Am Coll Cardiol*. 2004;43:1399–1404.
24. **Fang ZY, Prins JB, Marwick TH.** Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. *Endocr Rev*. 2004;25:543–567.
25. **Herring MJ, Oskui PM, Hale SL, Kloner RA.** Testosterone and the cardiovascular system: a comprehensive review of the basic science literature. *J Am Heart Assoc*. 2013;2:e000271.
26. **Corona G, Rastrelli G, Vignozzi L, Mannucci E, Maggi M.** Testosterone, cardiovascular disease and the metabolic syndrome. *Best Pract Res Clin Endocrinol Metab*. 2011;25:337–353.
27. **Green-Golan L, Yates C, Drinkard B, VanRyzin C, Eisenhofer G, Weise M, Merke DP.** Patients with classic congenital adrenal hyperplasia have decreased epinephrine reserve and defective glycemic control during prolonged moderate-intensity exercise. *J Clin Endocrinol Metab*. 2007;92:3019–3024.
28. **Ferrannini E, Cushman WC.** Diabetes and hypertension: the bad companions. *Lancet*. 2012;380:601–610.
29. **Minetto MA, Lanfranco F, Motta G, Allasia S, Arvat E, D'Antona G.** Steroid myopathy: some unresolved issues. *J Endocrinol Invest*. 2011;34:370–375.
30. **Sartorato P, Zulian E, Benedini S, Mariniello B, Schiavi F, Bilora F, Pozzan G, Greggio N, Pagnan A, Mantero F, Scaroni C.** Cardiovascular risk factors and ultrasound evaluation of intima-media thickness at common carotids, carotid bulbs, and femoral and abdominal aorta arteries in patients with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2007;92:1015–1018.
31. **Falhammar H, Filipsson H, Holmdahl G, Janson PO, Nordenskjold A, Hagenfeldt K, Thoren M.** Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2007;92:110–116.
32. **Cutler GB, Jr., Laue L.** Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *N Engl J Med*. 1990;323:1806–1813.
33. **de Silva KS, Kanumakala S, Brown JJ, Jones CL, Warne GL.** 24-hour ambulatory blood pressure profile in patients with congenital adrenal hyperplasia–a preliminary report. *J Pediatr Endocrinol Metab*. 2004;17:1089–1095.
34. **Volkl TM, Simm D, Dotsch J, Rascher W, Dorr HG.** Altered 24-hour blood pressure profiles in children and adolescents with classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab*. 2006;91:4888–4895.
35. **Muiesan ML, Lupia M, Salvetti M, Grigoletto C, Sonino N, Boscaro M, Rosei EA, Mantero F, Fallo F.** Left ventricular structural and functional characteristics in Cushing's syndrome. *J Am Coll Cardiol*. 2003;41:2275–2279.
36. **Tajima T, Ma XM, Bornstein SR, Aguilera G.** Prenatal dexamethasone treatment does not prevent alterations of the hypothalamic pituitary adrenal axis in steroid 21-hydroxylase deficient mice. *Endocrinology*. 1999;140:3354–3362.
37. **Ten S, New M, Maclaren N.** Clinical review 130: Addison's disease 2001. *J Clin Endocrinol Metab*. 2001;86:2909–2922.
38. **Bergthorsdottir R, Leonsson-Zachrisson M, Oden A, Johannsson G.** Premature mortality in patients with Addison's disease: a population-based study. *J Clin Endocrinol Metab*. 2006;91:4849–4853.
39. **Wang M, Yip GW, Wang AY, Zhang Y, Ho PY, Tse MK, Lam PK, Sanderson JE.** Peak early diastolic mitral annulus velocity by tissue Doppler imaging adds independent and incremental prognostic value. *J Am Coll Cardiol*. 2003;41:820–826.
40. **Allison TG, Cordeiro MA, Miller TD, Daida H, Squires RW, Gau GT.** Prognostic significance of exercise-induced systemic hypertension in healthy subjects. *Am J Cardiol*. 1999;83:371–375.