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Circadian blood pressure profiles and ambulatory arterial stiffness index in children and adolescents with congenital adrenal hyperplasia due to 21-hydroxylase deficiency in relation to their genotypes

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Abstract

OBJECTIVE: Lifelong steroid therapy and exposure to adrenal androgen excess in 21-hydroxylase deficient (21-OHD) congenital adrenal hyperplasia (CAH) children and adolescents may modify circadian blood pressure profile and result in vascular complications. The objective of the study was to evaluate vascular abnormalities in 21-OHD children and adolescents in relation to their genotypes.
DESIGN: A cross-sectional study conducted at a tertiary referral center.
Patients: Seventy patients with 21-OHD CAH (27 boys), aged from 3 to 17.9 years: 9 with nonclassic CAH, 61 with classic CAH: 10 with simple virilising (SV) and 51 with salt wasting CAH (13-Del/Del, 8-Del/I2G, 7-I2G/I2G and 23-other genotypes).
MAIN OUTCOMES MEASURES: The assessment of systolic and diastolic BP (SBP, DBP) loads, night dip% and arterial ambulatory stiffness index (AASI) in 21-OHD CAH patients.

ORIGINAL ARTICLE

RESULTS: The highest percentage of abnormal SBP loads was found in SW CAH patients with Del/Del genotype and DBP loads in SV CAH patients. The lowest percentage of abnormal SBP and DBP loads was found in NC CAH and in SW CAH I2G/I2G subgroup. Abnormal values of night time dip% and the highest values of AASI were found in Del/Del and Del/I2G. Girls were more affected than boys in relation to abnormal ABPM profiles. ABPM parameters were associated with cortisol values. AASI correlated positively with free androgen index.

CONCLUSION: Pediatric patients with CAH present vascular abnormalities related to the steroid therapy and androgen excess and pronounced more in certain subgroups of CAH (SV and SW: Del/Del, Del/I2G).

Abbreviations:

ABPM	- Ambulatory Blood Pressure Monitoring
AASI	- Ambulatory Arterial Stiffness Index
BP	- Blood Pressure
CAH	- Congenital Adrenal Hyperplasia
24 h SBP load	- 24 hour mean systolic BP load
24 h DBP load	- 24 hour mean diastolic BP load
dDBP	- mean day-time diastolic BP
dSBP	- mean day-time systolic BP
nDBP	- mean night-time diastolic BP
nSBP	- mean night-time systolic BP
FAI	- free androgen index
TST	- testosterone
SHBG	- sex hormone binding globulin
FM%	- fat mass%
LTM%	- lean tissue mass%
TBW%	- total body water%
FC	- Fludrocortisone
HC	- Hydrocortisone

INTRODUCTION

Lifelong steroid therapy and exposure to adrenal androgen excess in 21-hydroxylase deficient (21-OHD) congenital adrenal hyperplasia (CAH) children and adolescents may result in vascular complications (Falhammar *et al.* 2015).

In 21-OHD CAH a deficit in corticosteroids leads to an increase in ACTH synthesis and stimulation of the adrenal cortex. The accumulation of precursors above the block shunted subsequently to the adrenal sex hormone pathway exposes patients to androgen excess. There is a classic form comprising the salt wasting variant (SW) manifested neonatally by severe salt loss and virilisation of external genitalia in females and the simple virilizing variant (SV) where salt loss is mild or absent and manifested by GnRH-independent precocious puberty (Merke & Bornstein 2005). The nonclassic (NC) variant is usually diagnosed with hyperandrogenism later in childhood or adolescence (Merke & Bornstein 2005). The goal of therapy is a replacement of steroids (glucocorticoids and mineralocorticoids) to prevent adrenal crisis and suppression of the abnormal secretion of adrenal androgens (Speiser *et al.* 2010). Vascular profiles of 21OHD patients on lifelong steroid therapy depend on the balance between steroid under- or overtreatment (Harrington *et al.* 2012; Wojcik *et al.* 2013; Subbarayan *et al.* 2014). Glucocorticoids, often given in supraphysiological doses may lead to hypertension (Bachelot *et al.* 2007). The most severe type of CAH is associated with more intensive steroid treatment, and that may be a secondary cause of hypertension and later cardiovascular problems. Longstanding undertreatment with elevation of adrenal androgens may also increase vascular mortality (Maggio & Basaria 2009).

CAH children often have disrupted circadian cortisol rhythm and in consequence blood pressure (BP) rhythms, factors that may have important long-term health implications (Volkl *et al.* 2006; Wojcik *et al.* 2013). An abnormal BP circadian rhythm, and in particular a non-dipping phenomenon is associated with increased cardiovascular risks including left ventricular hypertrophy (Fumo *et al.* 1992; Sihm *et al.* 1995; Verdecchia *et al.* 1995), cerebrovascular (Kario *et al.* 1996) and cardiovascular morbidity (Verdecchia *et al.* 1995), kidney damage (Timio *et al.* 1995) and increased mortality (Ohkubo *et al.* 1997; Ohkubo *et al.* 2002; Wojcik *et al.* 2013).

The ambulatory arterial stiffnes index (AASI) is an indirect arterial stiffness measure, which can be derived from 24 hr ambulatory blood pressure monitoring (ABPM) and has been proven to be independently associated with cardiovascular adverse events, especially stroke (Xu *et al.* 2011; Kollias *et al.* 2012; Wojcik *et al.* 2015; Verbakel *et al.* 2016). In recent work Falhammar *et al.* (2015) analysing 588 CAH patients (>80% with known CYP21A2 mutations) from the national swedish population-based registers for the first time showed that in CAH population not only risk factors for cardiovascular disorders but also cardiovascular diseases were increased: hypertension, atrial fibrillation, venous thromboembolism and stroke.

There is a good phenotype-to-genotype correlation in 21OHD CAH and it seems that genotyping may be useful in predicting vascular risks in 21-OHD CAH patients (Falhammar *et al.* 2007; Hagenfeldt *et al.* 2008; Nordenskjold *et al.* 2008; Falhammar *et al.* 2009; Frisen *et al.* 2009; New *et al.* 2013; Falhammar *et al.* 2015).

The objective of the study was to evaluate circadian blood pressure profiles and ambulatory arterial stiffness index in 21OHD children and adolescents in relation to their genotypes.

SUBJECTS AND METHODS

Subjects

Seventy patients with 21-OHD CAH (27 boys), aged from 3 to 17.9 years: 9 with nonclassic CAH, 61 with classic CAH: 10 with simple virilising (SV) and 51 with salt wasting CAH (13-Del/Del, 8-Del/I2G, 7-I2G/I2G and 23-other genotypes). The patients were not diagnosed by newborn screening as it was unavailable at the time of the study. SW CAH patients were diagnosed in the neonatal period. SV patients were diagnosed at around 3±1 years of age and NC CAH patients at around 7±2 years of age. In all patients CAH was diagnosed in steroid urine profile. The diagnosis of 21OHD was confirmed by genotype in 56 patients. With regard to the remaining 14 cases all patients were diagnosed clinically with salt wasting CAH in the neonatal period and 21OHD was confirmed in steroid urine profile.

Patients with nonclassic form were receiving only hydrocortisone (HC) (mean dose 11.9±3.5 mg/m^2, tid) and all classic CAH patients received glucocorticoid replacement therapy with hydrocortisone (mean dose 17.2±4.2 mg/m^2 in SW CAH and 19.5±2.5 mg/m^2 in SV CAH,tid) and mineralocorticoid therapy with fludrocortisone (FC) (66.5±36.5 mcg/m^2 in SW CAH and 28.6±15.5 mg/m^2 in SV CAH, bid). The adequacy of therapy was monitored periodically on the basis of clinical and laboratory data, in accordance with current guidelines (Speiser *et al.* 2010). None of the patients used additional medication.

This study was conducted in accordance with the guidelines in The Declaration of Helsinki and was approved by the local ethical committee. All participants gave their informed consent.

Clinical study

Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using a stadiometer (Harpenden, UK) and a balanced scale. As the standard of reference normal values from the local population were used (Palczewska & Niedzwiedzka 2001).

Assays

Plasma cortisol, testosterone (TST) and sex hormone binding globulin (SHBG) were assessed by chemiluminescence immunoassay (Centaur-Bayer). Free androgen index (FAI) was calculated with the use of a formula: FAI=TST (nmol/l) × 100 /SHBG (nmol/l), the FAI norm is <5. Serum concentrations of upright aldosterone, plasma renin activity and 17-OHP were measured by RIA. Cortisol was assessed in plasma 2 hours after 1st, 2nd and 3rd dose of hydrocortisone. 24 hour collection of urine was used for the assessment of free cortisol in urine. The area under the curve was calculated with the use of formula:

$$A=\sum_{i=1}^{n}\frac{1}{2}h_i(a_i+b_i)$$

Bioimpedance analysis

Electrical bioimpedance analysis (BIA) was performed in patients using Nutriguard Data Input device with Bianostic electrodes (Fresenius BCM, Data Input, Germany). Following parameters were calculated: total body water (TBW%), lean tissue mass (LTM%) and fat mass (FM %).

ABPM assessment

24-hour BP monitoring was performed using an Ambulatory BP Monitor (Spacelabs 90217, USA) with methodology described by our group previously (Wojcik *et al.* 2013). The following parameters were analyzed: systolic and diastolic mean, day and night loads (24h SBP load%, dSBP, nSBP, 24hDBP load%, dDBP, nDBP), nocturnal dipping (nighttime dip%) and ambulatory arterial stiffness index (AASI). BP load was defined as the percentage of valid ambulatory BP measurements above a set threshold (95th percentile for sex and the height) value (Wühl *et al.* 2002; National High Blood Pressure Education Program Working Group 2004). Loads in excess of 30% were considered elevated. The calculation of nocturnal dipping was based on a formula by the American Heart Association: [(dSBP–nSBP)/dSBP] × 100. Normal dipping was defined as a ≥10% decline in BP (de Silva *et al.* 2004). AASI was derived from ABPM using a previously described formula (Kollias *et al.* 2012).

Statistical analysis

In order to compare the two groups the two-sided Mann-Whitney U-test and ANOVA tests were used. Spearman ρ was used to measure the strength of association between pairs of variables. The level of significance was set at $p<0.05$. Calculations were performed using the STATISTICA 10.0 PL soft ware (Poland).

RESULTS

Circadian blood pressure profiles in relation to genotypes are presented in Table 1. There were no significant differences in relation to age and BMI SDS between the subgroups of patients.

The highest HC doses were used in SV CAH and the lowest in NC CAH. The highest FC doses were used in SW Del/Del group and the lowest in SV CAH. In children with NC CAH we were not using FC. In SW CAH group the lowest doses of HC and FC were used in I2G/I2G subgroup.

Levels of plasma renin activity and aldosterone were related to CAH phenotype.

Studied CAH patients did not have an overt hypertension. 24hr SBP loads and day SBP loads were higher in Del/Del than in other subgroups. Night SBP load was higher in Del/Del and Del/I2G than in other genotypes. The lowest 24 hr SBP loads as well as day and night SBP loads were found in NC and I2G/I2G subgroups.

24 hr DBP, day DBP and night DBP loads were higher in SV CAH and lower in I2G/I2G, Del/I2G and NC CAH when compared to other groups.

AASI was higher in Del/Del and Del/I2G and lower in NC CAH when compared to other subgroups.

Abnormal night time dip was found in 57% of patients (data not shown). This parameter differed significantly between Del/Del and Del/I2G vs I2G/I2G (6.0±1.6 and 6.9±4.4 vs 12.4±5.2%).

Tab. 1. Circadian blood pressure profiles in CAH patients depending on genotype. Data are expressed as mean [SD].

CAH	NC	SV	SW					*p*-value
			Del/Del	Del/I2G	I2G/I2G	Other	Total	
N	9	10	13	8	7	23	51	
Age years	8.7[4.1]	11.7 [4.7]	8.2[4.2]	8.7[4.1]	6.7[3.2]	9.5[4.5]	8.7[4.9]	NS
BMI SDS	0.45 [1.1]	0.37 [1.1]	0.4 [1.3]	0.5 [1.4]	0.7 [1.1]	0.8 [2.1]	0.6 [1.6]	NS
Hydrocortisone daily dose (mg/m^2)	11.9 [3.5]abcfg	19.5 [2.5]ae	16.6 [3.7]c	18.9 [5.2]b	15.6 [5.1]e	17.1 [3.9]f	17.2 [4.2]g	<0.05
Fludrocortisone daily dose (mcg/m^2)	0	28.6 [15.5]adefg	79 [45.1]a	65.9 [30.2]d	64.5 [43.1]e	64.1 [31.9]f	66.5 [36.5]g	<0.05
PRA (ng/ml/h) [n:1.5-5.7]	2.1 [0.7]bg	2.7 [2.3]a	6.8 [5.2]abef	3.1 [1.1]	4.1 [1.7]e	3.5 [2.1]f	4.3 [2.1]g	<0.05
Aldosteron (pg/ml) [n:35-310]	169.6 [75.5]	177.9 [45.1]adef	87.1 [52.1]a	89.8 [45.1]d	94.1 [30.1]e	108.0 [88.7]f	96.6 [22.3]	<0.05
24h SBP load %	6.8 [8.5]bfg	16.8 [14.9]a	28.1 [15.5]abcd	13.3 [7.7]d	6.7 [5.2]c	20.4 [8.5]f	19.1 [21.2]g	<0.05
dSBP load %	4.6 [1.1]bfg	14.4 [13.5]	22.7 [11.1]bde	8.8 [5.5]d	8.2 [5.7]e	18.6 [20.8]f	16.5 [5.5]g	<0.05
nSBP load %	14.8 [8.8]b*	25.9 [12.2]a	38.6 [44.7]c*	30.4 [15.2]d	0 [0.0]abdce	23.5 [15.5]e	26.0 [10.1]	<0.05
24h DBP load %	6.9 [7.3]acfg	16.3 [9.7]ad	14.5 [13.1]c	6.9 [5.5]d*	8.1 [6.3]	14.8 [5.8]f*	12.3 [5.5]g	<0.05
dDBP load %	3.5 [2.1]afg	13.7 [5.5]ad	10.2 [5.6]	5.3 [2.2]d*	7.0 [3.2]	13.5 [5.6]f*	10.2 [5.3]g	<0.05
nDBP load (%)	18.7 [5.4]	27.3 [15.5]ae	25.4 [19.2]	13.9 [2.8]a	12.6 [8.6]e	20.0 [9.9]	19.4 [4.6]	<0.05
AASI	0.309 [0.1]bcg	0.337 [0.1]a	0.41 [0.1]b	0.47 [0.2]acef	0.339 [0.1]e	0.37 [0.1]f	0.399 [0.2]g	<0.05
Nighttime dip%	8.6 [3.8]	8.9 [4.1]	6.0 [1.6]c	**6.9** [4.4]d	12.4 [5.2]cdf	8.2 [4.9]f	7.7 [2.5]	<0.05
TBW%	55.1 [5.7]	53.9 [4.7]a	56.9 [6.1]	57.2 [5.0]a	55.4 [4.8]	54.4 [5.5]	55.8 [4.9]	<0.05

Symbols [a–g] present significant differences between two subgroups (the same letter). Legend: NC – non classic CAH, SV – simple virilising CAH, SW – salt wasting CAH, PRA – plasma renin activity.

TBW% was higher in Del/Del (not significantly) and Del/I2G and lower in SV CAH.

Daily cortisol profiles are presented in Table 2. The highest values of cortisol area under the curve and 24 hr urine cortisol were observed in SV CAH and the lowest in NC CAH and SW I2G/I2G.

Analysis of cortisol profiles revealed that morning levels of cortisol assessed 2 hours after the first dose of hydrocortisone were within the normal range [50–230 ng/ml]. In SV CAH group morning levels of cortisol were close to upper normal range. Later during the day we were able to reach a reduction of cortisol but we were unable to reach a good evening drop of cortisol mimicking a normal cortisol daily rhythm. The best cortisol profiles with lowest values of evening cortisol were reached in NC CAH group and also in I2G/I2G subgroup.

The highest level of FM% and lowest LTM% were found in SV CAH and opposite results were found in Del/I2G SW CAH.

The highest levels of TST and FAI and the lowest levels of SHBG were found in SV CAH. Opposite results were found in I2G/I2G subgroup. 17OHP values did not differ between the subgroups (data not shown).

Circadian blood pressure and cortisol profiles in CAH patients depending on gender are presented in Table 3. In all CAH forms females were presenting higher SBP loads than males (significance only in SW CAH group). DBP loads were higher in females than in males in NC and SV groups and night DBP load also in SW group (tendencies). AASI was higher and night dip was lower in females than males in NC (tendency) and SW groups. There were no differences in HC, FC, PRA, Aldosteron, cortisol metabolism parameters between

Tab. 2. Cortisol daily profiles in CAH patients depending on genotype. Data are expressed as mean [SD].

CAH	NC	SV	SW					*p*-value
			Del/del	Del/I2G	I2G/I2G	other	Total	
N	9	10	13	8	7	23	51	
cortisol area under the curve	382.1 [110.6]a	566.8 [157.2]acdefg	442.1 [138.2]c	429.4 [92.5]d	371.4 [118.6]e	415.7 [102.6]f	419.6 [103.4]g	<0.05
24hr urine cortisol (mcg/volume)	56.3 [44.5]a	161.8 [76.1] acdefg	64.4 [54.1]c	98.6 [54.2]d	54.2 [29.8]e	76.1 [64]f	74.1 [65]g	<0.05
Cortisol 2 hrs after 1st HC dose (ng/ml)	139.2 [72.6]a	219.5 [110.1]aefg	178.7 [71.7]c	178.5 [71.9]	140.9 [38.0]e	140.5 [51.6]fc	156.6 [61.4]g	<0.05
Cortisol 2 hrs after 2nd HC dose (ng/ml)	120.1 [71.9]a	172.9 [62.3]acdefg	135.8 [51.8]c	128.9 [34.5]d	118.2 [60.1]e	125.6 [38.2]f	128.1 [43.6]g	<0.05
Cortisol 2 hrs after 3rd HC dose (ng/ml)	78. [45.9]ab*	162.7 [56.6]acdefg	124.3 [59.4]cb	127.2 [37.9]d*	105.8 [59.5]e	128.5 [55.5]f	124.8 [53.3]g	<0.05
LTM%	70.0 [9.8]	68.9 [8.9]a	72.4 [11.1]	73.8 [7.1]a	69.9 [9.3]	69 [8.9]	71.1 [8.9]	<0.05
FM%	22.4 [8.4]	23.8 [6.8]a	20.1 [8.8]	19.3 [6.7]a	22.1 [6.6]	23.3 [7.9]	21.4 [6.5]	<0.05
TST (ng/ml)	0.4 [0.3]af	1.7 [1.2]aecdg	0.6 [0.1]c	0.6 [0.2]d	0.1 [0.1]e	1.3 [0.2]f	0.9 [0.2]g	<0.05
SHBG (nmol/l)	99.4 [10.4]	45.6 [5.4]ade	94.5[9.8]	109.0 [12.2]d	112.6 [12.5]e	77.8 [8.9]	93.9 [12.1]a	<0.05
FAI	2.29 [1.1]a	29.1 [5.4]acdefg	3.1 [1.1]c	3.2 [1.2]d	0.5 [0.0]e	7.1 [1.2]f	4.5 [1.0]g	<0.05

Symbols [a–g] present significant differences between two subgroups (the same letter). Legend: NC – non classic CAH, SV – simple virilising CAH, SW – salt wasting CAH.

the sexes apart from cortisol values after the 1st dose higher in SW CAH females. Testosterone, FAI and TBW% were higher in females than males in SW group.

Circadian blood pressure profiles in relation to bone age are presented in Table 4. There were no significant differences in age and BMI between groups with/without advanced bone age.

24 hr, day and night SBP loads% were significantly higher in patients with advanced bone age. There was a gender difference seen in SBP loads: 24 hr,day and night SBP were higher in females than males (significance in a group with advanced bone age). There were no differences in 24 hr DBP loads and day DBP loads between the groups. Significantly higher night DBP load was found in females with advanced bone age.

Night dip% did not differ between the groups,but females within both groups had night dipping lower than males. Females without advanced bone age had night dipping lower than females with advanced bone age.

AASI was higher in patients with advanced bone age. In males with advanced bone age AASI was significantly higher than in males without advanced bone age. There were no gender differences in AASI in a group with advanced bone age. AASI was higher in females than males in the group without advanced bone age.

Daily cortisol profiles in relation to bone age are presented in Table 5. There were no significant differences in cortisole profiles,17OHP and HC dose between groups with /without advanced bone age.

Cortisol in 24 hr urine collection, TST level and FM % were significantly higher and LTM%, TBW% and FC dose significantly lower in a group with advanced bone age.

In females without advanced bone age FC dose, TBW%, LTM% were higher and FM% lower than in females with advanced bone age. Lower cortisol area under the curve,cortisol in urine and more optimal cortisol profiles were found in females without advanced bone age than in females with advanced bone age. In both groups cortisol values in plasma and urine were lower in females than in males. There were no gender differences in relation to TST levels within both groups.

DISCUSSION

In the present study we aimed to assess for the first time the genotype-vascular correlations in our cohort of 21-hydroxylase deficient pediatric patients.

Studied CAH patients did not have an overt hypertension on a 3–4-monthly routine out-patient visits however 24-hour ABPM revealed a tendency to abnor-

Tab. 3. Circadian blood pressure and cortisol profiles in CAH patients depending on gender. Data are expressed as mean [SD].

CAH	NC		SV		SW		*p*-value
	M	F	M	F	M	F	
N	4	5	5	5	20	15	
Age years	8.1[2.8]	8.5[4.3]	12.1[5.7]	11.2[3.9]	6.7[4.0]	7.1[5.2]	Ns
HC mg/m2	11.3 [2.9]	12.8 [3.9]	20.7 [2.8]	18.3 [1.9]	17.7 [3.4]	16.7 [4.8]	Ns
FC mcg/m2	0	0	29.5 [35.5]	27.8 [30.6]	74.4 [41.9]	61.8 [29.1]	Ns
Cortisol area under the curve	413.9 [155.2]	358.1 [81.4]	669.1 [172.9]	485.1 [90.8]	439.9 [116.2]	403.0 [105.4]	Ns
Cortisol 2 hrs after 1st HC dose (ng/ml)	176.4 [107.5]	111.2 [20.7]	270.8 [131.3]	168.2 [59.3]	191.8 [58.4]*	142.9 [61.7]*	<0.05
Cortisol 2 hrs after 2nd HC dose (ng/ml)	80.8 [13.1]	149.2 [86.7]	199.7 [60.3]	146.2 [57.6]	122.7 [45.0]	137.2 [42.3]	Ns
Cortisol 2 hrs after 3rd HC dose (ng/ml)	100.9 [10.0]	61.2 [57.0]	181.5 [75.0]	147.7 [39.3]	134.9 [54.7]	116.6 [51.9]	Ns
PRA (ng/ml/h)	1.7[1.0]	2.1[0.9]	1.45[1.1]	3.9[2.6]	4.6[2.3]	3.1[1.2]	Ns
Aldosteron (pg/ml)	170.3 [13.3]	169 [173.9]	213.8 [270.0]	141.9 [112.0]	117.2 [120.7]	82.2 [92.6]	Ns
24h SBP load %	1.8[3.1]	10.5[9.7]	9.7[11.5]	23.9[15.5]	11.5[11.6]*	24.9[24.7]*	<0.05
dSBP load %	0.9[1.6]	7.4[7.9]	9.2[12.2]	19.5[14.0]	10.5[10.9]*	21.2[25.1]*	<0.05
nSBP load %	3.5[6.1]	23.3[27.7]	13.3[13.1]	38.5[29.2]	16.8[21.4]*	33.2[32.2]*	<0.05
24h DBP load %	6.9[10.9]	6.9[5.2]	10.7[8.6]	21.8[7.8]	13.6[15.5]	11.6[10.4]	Ns
dDBP load %	3.2[4.3]	3.7[5.4]	8.5[7.8]	18.7[8.7]	13.6[15.7]	11.6[7.8]	Ns
nDBP load %	14.0[24.0]	22.2[20.9]	21.7[13.9]	32.8[16.5]	18.4[22.9]	20.0[23.5]	Ns
AASI	0.21[0.1]	0.38[0.1]	0.35[0.1]	0.32[0.1]	0.38[0.1]*	0.42[0.2]*	<0.05
Nighttime dip%	11.8[3.0]	6.1[1.9]	8.9[2.4]	9.1[5.6]	10.0[4.2]*	6.01[8.9]*	<0.05
TST (ng/ml)	0.2[0.1]	0.5[0.3]	2.4[2.1]	0.7[0.5]	0.7[0.7]*	1.04[0.7]*	<0.05
FAI	0.6[0.4]	5.7[1.2]	41.9[28.3]	3.4[0.1]	4.4[5.6]*	4.6[4.2]*	<0.05
TBW%	58.5[7.5]	52.9[4.4]	55.1[4.6]	51.9[5.03]	54.4[5.7]*	57.4[3.8]*	<0.05

Symbol [*] presents significant differences between two subgroups. Legend: NC – non classic CAH, SV – simple virilising CAH, SW – salt wasting CAH

mal SBP and DBP loads mostly at nighttime and in more than 50% of patients abnormal night time dip. Interestingly we have found a positive correlation between all assessed parameters of 24h ABPM and cortisol level after the 3rd dose of HC. The highest percentage of abnormal SBP loads was found in SW CAH patients with Del/Del genotype and DBP loads in SV CAH patients. The lowest percentage of abnormal SBP and DBP loads was found in NC CAH and in I2G/I2G SW subgroup and in these two groups cortisol profiles, cortisol in urine and area under the curve were better mimicking the physiology. NC CAH patients were not receiving FC and in I2G/I2G FC and HC dose were lower than in other SW subgroups (a tendency). Del/Del patients were receiving the highest FC dose when compared to other subgroups, however the mean PRA was above the upper normal range.According to the current guidelines (Speiser *et al.* 2010) we were trying to avoid suppressing plasma renin activity below the lower normal range with FC.

AASI was highest in Del/Del and Del/I2G genotypes and lowest in NC CAH. AASI correlated with cortisol in urine, cortisol area under the curve and cortisol after the 1st dose of HC. The lowest night time dipping was found in Del/Del and Del/I2G and the highest in I2G/I2G. The meaning of abnormal ABPM results in Del/

Tab. 4. Circadian blood pressure profiles in CAH patients in relation to bone age. Data are expressed as mean [SD].

	Bone age advanced N=34			Bone age not advanced N=35			*p*-value
	M n=12	F N=16	Total	M N=14	F N=12	Total	
Age years	8.5[4.2]	10.8[4.0]	9.6[4.2]	7.8[4.7]	10.1[5.5]	8.3[5.1]	n.s.
Bone age years	10.8[4.1]	12.7[4.2]b	11.7[4.1]c	6.8[4.6]b	9.3[5.5]	7.6[5.1]c	<0.05
BMI SDS	0.8[1.4]	0.7[0.8]	0.4[1.3]	0.07[1.2]	0.06[1.4]	0.7[1.8]	n.s.
AASI	0.4[0.1]a	0.4[0.1]	0.38[0.1]c	0.3[0.2]da	0.4[0.2]d	0.37[0.2]c	<0.05
24 h SBP load%	10.9[10.0]a	22.3[20.7]ad	20.4[5.5]c	9.3[5.5]d	17.84[7.4]	13.4[7.1]c	<0.05
dSBP load%	10.5[11.6]a	22.3[24.5]abe	17.6[20.9]c	8.2[10.3]b	13.8[19.0]e	10.9[15.1]c	<0.05
nSBP load%	12.4[4.8]ae	36.8[30.4]adb	27.2[27.7]c	16.2[22.2]d	25.4[30.2]eb	20.6[26.3]c	<0.05
24 h DBP load%	10.7[4.5]	13.7[3.2]	13.1[3.7]	12.3[3.2]	9.7[9.5]	11.7[4.2]	n.s.
dDBP load%	10.1[13.8]	10.7[8.4]	10.5[10.6]	11.6[14.2]	6.9[8.8]	9.4[11.9]	n.s.
nDBP load%	13.5[18.8]ad	23.7[22.7]a	19.7[21.5]	22.3[22.3]d	16.8[16.9]	19.6[19.7]	<0.05
Night dip %	10.7[4.5]a	8.3[4.5]b	9.2[4.6]	9.4[3.3]d	4.5[10.9]abd	7.0[8.2]	<0.05
PRA (ng/ml/h) [n:1.5-5.7]	3.0[4.1]a	2.9[3.5]b	2.9[3.7]	5.4[7.5]abe	2.9[3.5]e	4.2[5.9]	n.s.
Aldosteron (pg/ml) [n:35-310]	186.2 [197.9]ad	124.8 [127.7]b	148.1 [157.5]	108.3 [100.7]d	71.0 [75.1]ab	88.9 [88.6]	<0.05

Symbols [a,b,d,e] present significant differences between two subgroups with the same letter. Legend: PRA – plasma renin activity.

Del and Del/I2G children is not known. According to Falhammar *et al.* (2015) even if SBP and DBP loads are higher in severe phenotypes as was also observed in presented study it is speculated that due to lower epinephrine production in SW Del/Del and I2G phenotype the risk for further cardiovascular events might be smaller than in milder phenotypes.

The prevalence of hypertension varies widely between studies. Some report systolic and diastolic hypertension (Roche *et al.* 2003;Merke & Bornstein,2005;Finkielstain *et al.* 2012;Amr *et al.* 2014;Subbarayan *et al.* 2014). Others in the paediatric CAH patients with normal weight even showed diastolic hypotension (Volkl *et al.* 2006). Ubertini *et al.* (2009) found that systolic and diastolic BP was normal in CAH patients. This might be due to the use of lower doses of fludrocortisone compared with our study (mean dose 48 vs 74.8 mcg/m2) as well.

Comparisons between the three CAH forms revealed that the SV CAH form (in 50% with I172N genotype) was the one most negatively affected in relation to metabolic parameters like increased TST, FAI, low SHBG, low lean muscle mass, high fat mass, decreased TBW% and increased diastolic pressure. It is in contrast to Subbarayan *et al.* (2014) who did not find significant difference in the prevalence of hypertension between SV and SW groups. Interestingly, we have found that in our study the mean glucocorticoid doses in SV CAH were higher than in SW and NC CAH groups. This is due to the goal of the therapy in this subgroup: to decrease hyperandrogenemia causing GnRH-independent precocious puberty. Unfavourable cortisol daily profile, area under the curve and cortisol in urine indicate that the doses of corticosteroids used in SV CAH form were too high considering the milder form of disease due to a higher activity of 21-hydroxylase than in SW subgroups (Falhammar *et al.* 2015). We could also speculate that the compliance might be better in precocious puberty in CAH but it certainly needs further research. As mean time for diagnosis in SV CAH was around 3 years of age probably prolonged androgen excess might also contribute to adverse metabolic effects. In SW patients Del/Del and Del/I2G subgroups were most negatively affected in relation to SBP loads, AASI and night time dipping what is related to higher FC doses and TBW% than in other subgroups.

Although these were mostly tendencies, they confirmed different metabolic profiles with a tendency to overtreatment and increase in fat mass in SV CAH and undertreatment with a tendency to salt wasting and increased lean tissue mass in SW CAH (especially Del/Del and Del/I2G).

The most favourable outcome in relation to metabolism and vascular assessments was found in NC CAH and I2G/I2G SW subgroup. The mean glucocorticoid

Tab. 5. Cortisol daily profiles in CAH patients in relation to bone age. Data are expressed as mean [SD]. Symbols [a,b,d,e] present significant differences between two subgroups with the same letter.

	Bone age advanced N=34			Bone age not advanced N=35			*p*-value
	M n=12	F N=16	Total	M N=14	F N=12	Total	
Cortisol area under the curve	482.2 [108.5]a	433.4 [92.5]b	451.5 [99.6]	467.6 [178.1]d	373.0 [112.8]abd	423.7 [156.2]	<0.05
24hr urine cortisol (mcg/volume)	102.9 [72.3]a	87.2 [72.6]	93.4 [71.6]c	96.1 [153.8]	61.3 [65.2]a	78.7 [117.3]c	<0.05
Cortisol 2 hrs after 1st HC dose (ng/ml)	205.4 [52.9]ae	143.5 [47.4]ab	167.8 [57.6]	181.9 [105.9]b	151.7 [71.8]e	167.9 [91.3]	<0.05
Cortisol 2 hrs after 2nd HC dose (ng/ml)	141.7 [60.8]	149.2 [51.9]b	146.3 [54.6]	126.0 [55.5]	117.3 [43.0]b	121.9 [49.4]	<0.05
Cortisol 2 hrs after 3rd HC dose (ng/ml)	125.8 [60.5]	114.8 [58.8]b	118.9 [58.5]	141.1 [57.4]bd	109.4 [4.2]d	127.0 [53.1]	<0.05
17OHP (ng/ml)	7.6[4.1]	8.01[3.5]	6.9[4.2]	5.7[5.1]	7.0[4.6]	6.4[5.0]	n.s.
TST (ng/ml)	1.3[1.5]	0.9[0.7]	1.12[1.1]c	0.8[1.2]	0.6[0.5]	0.7[0.9]c	<0.05
FAI	22.5[20.1]ab	6.32[4.3]b	13.7[5.4]c	8.6[4.5]	1.7[1.6]a	5.4[3.0]c	<0.05
SHBG (nmol/l)	61.4 [49.4]	65.2 [43.3]	65.2 [43]c	102.7 [58.9]	103.5 [50.6]	100.7 [50.2]c	<0.05
HC dose (mg/m^2)	17.9[4.4]	17.3[5.5]	17.6[4.9]	16.5[3.6]	16.3[3.8]	16.4[3.7]	n.s.
FC dose (mcg/m^2)	45.0[12.1]a	39.1[32]be	41.6[40.4]c	69.2[42.0]ab	59.9[36.3]e	66.4[38.1]c	<0.05
LTM%	69.5[11.2]a	66.3[6.9]b	67.7[8.7]c	71.5[7.1]e	78.1[4.8]abe	73.7[6.9]c	<0.05
FM%	22.8[9.4]a	25.3[5.4]b	24.2[7.2]c	21.5[6.1]e	15.9[3.7]abe	19.6[5.9]c	<0.05
TBW%	54.9[5.2]a	53.1[5.2]b	53.6[5.2]c	55.4[4.6]e	59.6[4.2]abe	56.9[4.4]c	<0.05

doses were lowest compared to other subgroups and subsequently cortisol daily profile, cortisol area under the curve and cortisol in urine were mimicking better the physiology. Additionaly NC CAH were not treated with FC and presented lower androgen levels. This is in contrast to study by Williams *et al.* (2010) where NC-CAH boys and girls had higher systolic blood pressure compared with controls, in contrast to classic CAH boys and girls. In I2G/I2G SW subgroup FC dose was lower than in other SW subgroups. If it means that I2G/I2G SW patients may have more favourable metabolic profile needs further studies. As presented by New *et al.* (2013) although in most cases I2G mutation in intron 2 is associated with the SW phenotype as it was in all our I2G/I2G patients diagnosed in neonatal period with salt wasting phenotype, some patients present with the SV form (New *et al.* 2013). It was observed that the I2G (g.655A/C>G) mutation activates a cryptic upstream 3' splice acceptor site and causes aberrant splicing and its occasional association with the SV form is probably due to the correct splicing of a small number of transcripts (New *et al.* 2013).

In our study the highest percentage of abnormal SBP loads was found in females in all groups (NC, SV and SW) and DBP in females in NC and SV group (significance only in SW group). Additionaly females in NC and SW groups had higher AASI and lower night dipping levels than males (significance only in SW group). Interestingly, in Falhammar *et al.* (2015) study, adult females were generally more affected especially in SV (I172N) and the nonclassic group than males, and that supports our study. Additionally Falhammar *et al.* (2015) presented an increased risk of stroke in NC females and in our study there was a tendency to higher AASI in females than males in NC group. Kollias et. al. (2012) in a meta-analysis and systematic review presented evidence suggesting that AASI, an indirect parameter of arterial function, independently predicts future cardiovascular events, particularly stroke.

In our study there were no gender differences in HC, FC dosing and BMI between males and females. It could be speculated that longer androgen exposure can increase the vascular risk in females. 24 hr SBP load and night time SBP load were positively correlated with

TST and FAI levels, suggesting that higher nocturnal SBP could represent an early effect of androgen excess similarly to Ubertini *et al.* (2009) study, who found correlation between mean DBP and nocturnal DBP and TST level.We found also an association between AASI as well as night dip% and FAI that could also confirm the negative influence of hyperandrogenism on arterial wall function especially in females.

In order to assess the effect of prolonged androgens excess on ABPM parameters we evaluated patients with and without advanced bone age. 24 hr SBP , day SBP and night SBP loads were significantly higher in patients with advanced bone age. There was a gender difference seen in SBP: 24 hr, day and night loads were higher in females than males (significance in a group with advanced bone age).

AASI was higher in patients with advanced bone age. In males with advanced bone age AASI was higher than in males without advanced bone age. Whether it means that androgen excess can increase cardiovascular risks (stroke) also in CAH males needs follow-up research. AASI was higher in females than males in the group without advanced bone age.

In females without advanced bone age higher: FC dose, TBW%, LTM%, lower: FM%, cortisol area under the curve,cortisol in urine and more optimal cortisol profiles were found than in females with advanced bone age. In both groups cortisol values in plasma and urine were lower in females than in males. There were no gender differences in relation to TST within both groups.

To summarise it seems that females are more affected than males by prolonged androgens exposure in a group with advanced bone age. In the group without advanced bone age it seems that AASI and BP parameters are influenced by FC dose causing an increase in TBW%. We have found a correlation between FC dose and TBW%, as well as between TBW% and SBP and DBP loads.

This cross-sectional study presents some limitations. The most important one is small number of patients in CAH subgroups for ascertaining associations however some of them have been in accordance to other large studies in adults assessing genotype-metabolic correlations (Falhammar *et al.* 2015). Another limitation is no control group. All comparisons were performed within the CAH subgroups.

CONCLUSION

In the examined group children and adolescents with CAH present vascular abnormalities related to the steroid therapy and androgen excess pronounced more in certain subgroups of CAH (SV, SW: Del/Del and Del/I2G) and in females. We have found a negative influence of androgens on BP parameters both in males and females. These single centre results might be encouraging to use genotyping in monitoring corticosteroid and FC dosing in pediatric CAH patients. Future larger multicenter studies are necessary to present genotype and metabolic correlations in children.

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Supplementary materials:

Correlations (all with p<0.05):

AASI correlates with SHBG (r:–0.4) , FAI (r:0.4), 17OHP (r:0.3), night SBP load (r:0.3), night dip (r:–0.5),HC dose (r:0.3), cortisol in urine (r:0.3), cortisol area under the curve (r:0.3), cortisol after the 1st dose of HC (r:0.4).

Night dip% correlates with FAI (r:–0.5).

24 hr SBP load% correlates with TST (r:0.3), SHBG(r:–0.4), FAI (r:0.4), cortisol 2h after 3rd dose of HC(r:0.3), TBW (r:0.4), LTM% (r:–0.4), FM% (r:0.4).

Day SBP load% correlates with cortisol 2h after 3rd dose (r:0.3), TBW (r:0.5), LTM% (r:–0.5), FM% (r:0,5).

Night SBP load% correlates with TST (r:0.3), SHBG(r:–0.3), FAI (r:+0.4), cortisol after the 3rd dose of HC (r:0.3), LTM% (r:–0.4).

24 DBP load% correlates with SHBG(r:–0.4), cortisol after the 3rd dose of HC(r:0.4), TBW (r:0.5), LTM% (r:–0.5), FM% (r:0.5).

Day DBP load% correlates with :cortisol area under the curve (r:0.3), cortisol after the 3rd dose of HC (r:0.5), TBW (r:0.5), LTM% (r:–0.5), FM% (r:0.5).

Night DBP load% correlates with SHBG (r:–0.4), cortisol after the 3rd dose of HC (r:0.3), TBW (r:–0.6),LTM% (r:–0.6), FM% (r:0.6).

FC dose correlates with TBW% (r:0.4, p<0.05).