# Supplementary Materials: Blood pressure and its associations in 554 children and young people with CAH

## Supplementary methods 1 – Full details of data preparation

Data was prepared for analysis by removal of duplicated entries, and manual assessment of outlying values using data visualisation, followed by contact with individual centres for clarification about possible data entry errors and units of measurement.

*Interpolation of missing height and weight data*

Due to varying number of visits within patients, a hierarchical approach was employed to interpolation of missing height and weight. In patients with 11 or more visits in which height and weight were available, a generalised additive model was estimated for height on age with weight as a covariate. Pearson scaled residuals were calculated for each data point within this fit to assess for outliers. Individual model fits were examined visually and an absolute Pearson scaled residual of 3 or more was selected to declare a datapoint as an implausible outlier allowing deletion of that point and imputation. If a datapoint was declared an outlier, the modelling was reiterated without the outlying point before establishing the final GAM model fit that was then used to interpolate missing or outlying data points. In patients with 11 or more visits in which height was available but the covariate weight was not available, the process was repeated to fit a GAM model without the covariate of weight.

In patients with between 4 and 10 known data points for height, a cubic spline with 4 degrees of freedom was fit through the data, without covariate adjustment. Residuals were calculated manually from the spline fit by calculating the difference between the model fit and each data point, and then standardising by dividing them by the standard deviation of the height measurements within that patient. A threshold of greater than 0.5 was used for this standardised residual to declare a data point as an outlier following visual inspection of the model fits. In patients with between 2 and 4 available data points for height, a linear interpolation method was employed. Points were not extrapolated beyond known data points.

A similar process was applied for measurements of weight, with different outlier threshold parameters due to the greater variability in this metric with true values able to decrease as well as increase. The residual threshold for GAM models was thus set at a pearson residual of 4, and for spline models a standardised residual threshold of 1.2.

*Imputation of missing dose data*

Missing data patterns were reviewed and correlated with other data fields of relevance. The field ‘has dose changed from previous visit’, evidenced that doses patients were prescribed at that visit going forward were entered into the registry, with a preference for dose changes to be entered into the registry: Patients who were maintained on the same dose were more likely to have their precise dose details missing, with dose changes more likely to include precise dosing details. A pragmatic approach of data imputation using last observation carried forward was therefore employed, with limits on how far data could be carried according to age, restricted to carrying for 6 months under age 1, 12 months under age 3, 24 months under age 5 and 36 months over age 5. If data was not available to carry forward, then next observation carried backward was employed with the same age limits. The dose a patient was on prior to clinic (as defined by the previous visit) was therefore used for modelling to assess the effect of that dose on the patient parameters measured in clinic. Hydrocortisone equivalent dosing was converted using the conversion factors in table S2.

*Joint modelling multiple imputation of BP and biomarkers*

The remaining variables of interest exhibited more random variation within patients, and were thus more suited to multiple imputation, as carrying values or interpolating values would result in less plausible random variation around the trend. These metrics were imputed using a joint modelling multiple imputation model with complete age, sex, interpolated height and interpolated weight used as covariates. Biomarkers were converted to consistent units (table S3) and natural log transformed prior to imputation to achieve a closer to normal shaped distribution. Imputation was carried out 10 times to create 10 different imputed datasets. Following imputation, the standardised lower and upper limits of detection (table S3) were applied to maintain consistency across the datasets and prevent the imputation of excessively large or small values that would have undue leverage on multivariable modelling. Biomarkers were censored to consistent lower and upper levels of detection to prevent undue leverage of high outlying values or extreme values created by log transforming values equal to or close to zero using the values in table S3.

This hybrid approach to missing data imputation was complicated, and tested by visualising the data and by rerunning multivariable models on complete case data without data imputation. Figure S2 shows a similar trend with age across all the variables, and the sensitivity analyses rerunning models on complete cases showed no change in the direction, and very little change in the magnitude of any of the estimates of interest (Table S8).

## Table S1 – R software packages employed during analysis

|  |  |
| --- | --- |
| **Name of software package** | **Citation** |
| Base R | https://www.R-project.org/ |
| tibble | https://tibble.tidyverse.org/ |
| rlang | https://cran.r-project.org/web/packages/rlang/index.html |
| dplyr | https://github.com/tidyverse/dplyr |
| doRNG | https://cran.r-project.org/web/packages/doRNG/index.html |
| piecewiseSEM | http://dx.doi.org/10.1111/2041-210X.12512 |
| lme4 | https://cran.r-project.org/web/packages/lme4/index.html |
| mice | https://cran.r-project.org/web/packages/mice/index.html |
| miceRanger | https://cran.r-project.org/web/packages/miceRanger/index.html |
| mgcv | https://cran.r-project.org/web/packages/mgcv/index.html |
| sjstats | https://cran.r-project.org/web/packages/sjstats/index.html |
| rlist | https://renkun-ken.github.io/rlist/ |
| pedsbp | https://cran.r-project.org/web/packages/pedbp/readme/README.html |
| ggplot2 | https://ggplot2.tidyverse.org |
| ggpubr | https://rpkgs.datanovia.com/ggpubr/ |
| gridExtra | https://cran.r-project.org/web/packages/gridExtra/ |
| DescTools | https://cran.r-project.org/package=DescTools |
| summarytools | https://cran.r-project.org/web/packages/summarytools/vignettes/introduction.html |
| Hmisc | https://cran.r-project.org/web/packages/Hmisc/index.html |
| rms | https://cran.r-project.org/web/packages/rms/index.html |
| mfp | https://cran.r-project.org/package=mfp |
| glmnet | https://cran.r-project.org/web/packages/glmnet/index.html |
| brms | https://cran.r-project.org/web/packages/brms/index.html |
| Shiny | http://www.rstudio.com/shiny/ |

## Table S2 – Conversion factors for glucocorticoid equivalents

Conversion factors to create hydrocortisone equivalent doses

|  |  |
| --- | --- |
| **Preparation of glucocorticoid** | **Multiplication factor to create hydrocortisone equivalent** |
| **Hydrocortisone** | 1 |
| **Prednisolone** | 4 |
| **Dexamethasone** | 80 |
| **Cortisone acetate** | 0.8 |
| **Methylprednisolone** | 5 |

## Table S3 – Conversion factors for biomarkers

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Marker** | **Original unit** | **Unit to**  **convert to** | **Multiplication factor** | **Standardised lower limit of detection** | **Standardised upper limit of detection** |
| **17OHP** | ng/ml | nmol/l | 0.030261 | 0.05nmol/l | 1000nmol/l |
| **Androstenedione** | ng/dl | nmol/l | 0.034916 | 0.05nmol/l | 100nmol/l |
| **Plasma Renin Activity (PRA)** | ng/ml/hr | nmol/l/hr | 0.77154 | *Converted to renin first* | *Converted to renin first* |
| **PRA to renin** | nmol/l/hr | µIU/ml | 0.158 | *Converted to renin first* | *Converted to renin first* |
| **Renin** | ng/l | µIU/ml | 1.67 | 0.05µIU/ml | 1000µIU/ml |

## Table S4 – Summary statistics with imputed values

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sex assigned at birth:** | **Male** | **Female** | **Not assigned** | **Total sample** |
| **Number of countries** | 17 | 17 | 4 | 18 |
| **Number of centres** | 31 | 31 | 4 | 35 |
| **Number of patients** | 257 | 291 | 6 | 554 |
| **Number of visits** | 3018 | 3361 | 57 | 6436 |
| **Number of visits per patient**  **Median (Q1 to Q3)** | 9  (7 to 16) | 9  (5 to 16) | 8  (2 to 11) | 9  (6 to 16) |
| **Number of years visits spanned within patients**  **Median (Q1 to Q3)** | 3.2  (2.7 to 6.9) | 3.2  (2.1 to 8.0) | 2.1  (0.2 to 3.1) | 3.2  (2.5 to 7.3) |
| **Age of patients at youngest visit (years)**  **Median (Q1 to Q3)** | 0.13  (0.04 to 0.99) | 0.21  (0.04 to 3.03) | 0.08  (0.03 to 0.26) | 0.16  (0.04 to 2.17) |
| **Age of patients at oldest visit (years)**  **Median (Q1 to Q3)** | 5.70  (3.09 to 11.47) | 6.01  (3.07 to 14.35) | 2.25  (0.46 to 3.17) | 5.81  (3.07 to 12.87) |
| **Systolic BP at visit (mmHg)**  **Median, (n) [Q1 to Q3]‡** | 107 (n=1556)  [97 to 118]  *105 (n=2936)*  *[96 to 116]* | 105 (n=1652)  [96 to 116]  *104 (n=3257)*  *[95 to 114]* | 99 (n=21)  [85 to 107]  *96 (n=53)*  *[85 to 105]* | 106 (n=3229)  [97 to 117]  *105 (n=6246)*  *[95 to 115]* |
| **Systolic BP SDS at visit‡**  **Median (n) [Q1 to Q3]** | 1.5 (n=1492)  [0.5 to 2.4]  *1.5 (n=2792)*  *[0.5 to 2.5]* | 1.1 (n=1564)  [0.3 to 2.1]  *1.2 (n=3080)*  *[0.3 to 2.2]* | - | 1.3 (n=3056)  [0.4 to 2.2]  *1.4 (n=5872)*  *[0.4 to 2.3]* |
| **Diastolic BP at visit (mmHg)**  **Median, (n) [Q1 to Q3]‡** | 64 (n=1542)  [57 to 71]  *64 (n=2936)*  *[57 to 71]* | 64 (n=1645)  [57 to 70]  *64 (n=3256)*  *[57 to 71]* | 60 (n=20)  [50 to 71.25]  *59 (n=53)*  *[50 to 69]* | 64 (n=3207)  [57 to 70]  *63.97 (n=6245)*  *[57 to 71]* |
| **Diastolic BP SDS at visit‡**  **Median (n) [Q1 to Q3]** | 1.1 (n=1478)  [0.4 to 2]  *1.3 (n=2792)*  *[0.5 to 2.2]* | 0.9 (n=1559)  [0.3 to 1.6]  *1.1 (n=3080)*  *[0.3 to 1.9]* | - | 1.0 (n=3037)  [0.3 to 1.8]  *1.2 (n=5872)*  *[0.4 to 2.1]* |
| **Visits prescribed hydrocortisone\***  **n (%) [missing n, % missing]‡** | 2200 (72.9%)  [610, 20.2%]  *2670 (88.5%)*  *[133 (4.4%)]* | 2214 (65.9%)  [648, 19.3%]  *2668 (79.4%)*  *[134 (4.0%)]* | 35 (61.4%)  [22, 38.6%]  *51 (89.5%)*  *[6 (10.5%)]* | 4449 (69.1%)  [1280, 19.9%]  *5389 (83.7%)*  *[273 (4.2%)]* |
| **Total Hydrocortisone equivalent at visit per BSA (mg/m2)†‡**  **Median (n) [Q1 to Q3]** | 14.1 (n= 2237)  [9.8 to 15.5]  *13.8 (n= 2774)*  *(9.7 to 15.2)* | 14.5 (n= 2438)  [9.9 to 15.7]  *14.1 (n= 3099)*  *(9.8 to 15.6)* | 18.4 (n= 25)  [13.5 to 20.3]  17.2 (n= 48)  (13.5 to 19.9) | 14.3 (n= 4700)  [9.9 to 15.6]  *14.0 (n= 5921)*  *(9.8 to 15.5)* |
| **Visits prescribed fludrocortisone**  **n (%) [missing n, % missing] ‡** | 2577 (85.4%) [180, 6.0%]  *2618 (86.8%) [125, 4.1%]* | 2754 (81.9%) [140, 4.2%]  *2804 (83.4%)*  *[77, 2.3%]* | 51 (89.5%)  [6, 10.5%]  *55 (96.5%)*  *[2, 3.5%]* | 5382 (83.6%) [326, 5.1%]  *5477 (85.1%) [204, 3.2%]* |
| **Total Fludrocortisone at visit per body surface area (when prescribed) (µg/m2) ‡**  **Median (n) [Q1 to Q3]** | 318 (n= 2442)  [103 to 396]  *322 (n= 2536)*  *[105 to 400]* | 292 (n= 2527)  [99 to 321]  *296 (n= 2693)*  *[99 to 323]* | 555 (n= 38)  [207 to 484]  *561 (n= 52)*  *[203 to 505]* | 307 (n= 5007)  [102 to 356]  *311 (n= 5281)*  *[103 to 359]* |
| **Visits prescribed salt**  **n (%), [missing n, % missing] ‡** | 400 (13.3%)  [290, 9.6%]  *400 (13.3%)*  *[265, 8.8%]* | 486 (14.5%)  [204, 6.1%]  *486 (14.5%)*  *[175, 5.2%]* | 35 (61.4%)  [5, 8.8%]  *35 (61.4%)*  *[4, 7.0%]* | 921 (14.3%)  [499, 7.8%]  *921 (14.3%)*  *[444, 6.9%]* |
| **Renin (µΙU/ml)**  **Median (n) [Q1 to Q3] ‡** | 5.0 (n=1059)  [0.3 to 69.1]  *9.2 (n=2945)*  *[0.9 to 75.2]* | 4.0 (n=1041)  [0.4 to 54.0]  *6.8 (n=3261)*  *[0.8 to 56.0]* | 0.4 (n=16)  [0.1 to 3.2]  *2.6 (n=53)*  *[0.2 to 38.2]* | 4.5 (n=2116)  [0.4 to 61.8]  *7.7 (n=6259)*  *[0.8 to 64.4]* |
| **17-ΟΗ Progesterone (nmol/ml)**  **Median (n) [Q1 to Q3] ‡** | 18.2 (n=1380)  [2.0 to 100.0]  *17.0 (n=2965)*  *[2.8 to 92.1]* | 26.9 (n=1325)  [3.0 to 140.0]  *20.93 (n=3279)*  *[2.96 to 117.61]* | 12.1 (n=31)  [4.0 to 62.8]  *15.94 (n=54)*  *[3.81 to 65.54]* | 21.18 (n=2736)  [2.72 to 115.0]  *18.87 (n=6298)*  *[2.95 to 102.84]* |
| **Androstenedione (nmol/ml)**  **Median (n) [Q1 to Q3] ‡** | 0.1 (n=1285)  [0.1 to 3.0]  *0.5 (n=2960)*  *[0.1 to 2.6]* | 1.0 (n=1211)  [0.1 to 6.0]  *1.0 (n=3270)*  *[0.1 to 5.0]* | 0.1 (n=29)  [0.1 to 1.0]  *0.2 (n=53)*  *[0.1 to 1.2]* | 0.7 (n=2525)  [0.1 to 3.5]  *0.8 (n=6283)*  *[0.1 to 3.6]* |

n=Number, BSA=Body surface area; Q1=Quartile 1; Q3=Quartile 3; SDS=Standard deviation score

‡Normal font, black text = original data; Italics, grey text = imputed data (mean of median and quartiles calculated on each imputed data set)

\*remaining visits patients prescribed either cortisone acetate (n=352), dexamethasone (134), prednisone (37), prednisolone (105), methylprednisolone (1), mixed dosing (59) or no glucocorticoid (86) (Table S5)

†Hydrocortisone equivalent calculated by multiplying preparations by the following factors: prednisolone/prednisone x4; dexamethasone x80; cortisone acetate x0.8; methylprednisolone x5 (Table S2 for full frequency tables of preparations)

## Table S5 – Time of day of biomarker measurements

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sex assigned at birth:** | **Male** | **Female** | **Not assigned** | **Total sample** |
| **Renin:** | | | | |
| Median (IQR) of measurements with time of reading (µIU/ml) | 1.6  (0.1 to 46.5) | 0.9  (0.1 to 12.4) | 0.4  (0.1 to 1.9) | 1.1  (0.1 to 32.5) |
| Number of measurements available that have time of reading (%) | 568  (53.6%) | 398  (38.2%) | 14  (87.5%) | 980  (46.3%) |
| Median (IQR) of time of reading (HH:MM) | 14:00  (09:30 to 15:45) | 13:51  (09:14 to 15:30) | 14:20  (13:51 to 14:40) | 13:58  (09:29 to 15:37) |
| Median (IQR) of measurements without time of reading (µIU/ml) | 13.0  (1.7 to 100.1) | 11.0  (2.0 to 95.5) | 3.0  (1.5 to 4.5) | 12.0  (1.8 to 99.2) |
| Number of measurements available that do not have time of reading (%) | 491  (46.4%) | 643  (61.8%) | 2  (12.5%) | 1136  (53.7%) |
|  |  |  |  |  |
| **17-OH Progesterone:** |  |  |  |  |
| Median (IQR) of measurements with time of reading (nmol/l) | 9.1  (1.3 to 44.0) | 6.1  (1.0 to 41.0) | 5.0  (3.0 to 40.5) | 8.0  (1.0 to 43.0) |
| Number of measurements available that have time of reading (%) | 672  (48.7%) | 498  (37.6%) | 19  (61.3%) | 1189  (43.5%) |
| Median (IQR) of time of reading (HH:MM) | 13:44  (08:00 to 15:21) | 13:43  (08:17 to 15:29) | 14:21  (13:59 to 14:43) | 13:45  (08:04 to 15:25) |
| Median (IQR) of those without time of reading (nmol/l) | 41.7  (3.9 to 146.5) | 59.5  (8.0 to 201.5) | 36.3  (8.3 to 114.2) | 48.4  (6.1 to 167.2) |
| Number of measurements available that do not have time of reading (%) | 708  (51.3%) | 827  (62.4%) | 12  (38.7%) | 1547  (56.5%) |
|  |  |  |  |  |
| **Androstenedione:** |  |  |  |  |
| Median (IQR) of measurements with time of reading (nmol/l) | 0.1  (0.1 to 1.4) | 0.1  (0.1 to 2.0) | 0.1  (0.1 to 0.1) | 0.1  (0.1 to 1.8) |
| Number of measurements available that have time of reading (%) | 589  (45.8%) | 402  (33.2%) | 19  (65.5%) | 1010  (40.0%) |
| Median (IQR) of time of reading (HH:MM) | 13:58  (08:14 to 15:39) | 13:41  (08:22 to 15:30) | 14:21  (13:59 to 14:43) | 13:56  (08:22 to 15:31) |
| Median (IQR) of measurements without time of reading (nmol/l) | 0.1  (0.1 to 3.5) | 1.0  (0.1 to 7) | 3.0  (0.5 to 7.4) | 1.0  (0.1 to 7.0) |
| Number of measurements available that do not have time of reading (%) | 696  (54.2%) | 809  (66.8%) | 10  (34.5%) | 1515  (60.0%) |

## Table S6 – Full frequency table of preparations of glucocorticoid prescribed at visit

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Preparation** | **All**  **visits** | **Male**  **visits** | **Female**  **visits** | **Visits sex not assigned** |
| **Hydrocortisone** | 5389  (83.7%) | 2670  (88.5%) | 2668  (79.4%) | 51  (89.5%) |
| **Prednisolone** | 105  (1.6%) | 12  (0.4%) | 93  (2.8%) | 0  (0.0%) |
| **Prednisone** | 37  (0.6%) | 12  (0.4%) | 25  (0.7%) | 0  (0.0%) |
| **Dexamethasone** | 134  (2.1%) | 36  (1.2%) | 98  (2.9%) | 0  (0.0%) |
| **Methylprednisolone** | 1  (0.1%) | 1  (0.1%) | 0  (0.0%) | 0  (0.0%) |
| **Cortisone Acetate** | 352  (5.5%) | 112  (3.7%) | 240  (7.2%) | 0  (0.0%) |
| **Mixed dosing** | 59  (0.9%) | 4  (0.1%) | 55  (1.6%) | 0  (0.0%) |
| **None** | 86  (1.3%) | 38  (1.2%) | 48  (1.4%) | 0  (0.0%) |
| **Unknown** | 273  (4.2%) | 133  (4.4%) | 134  (4.0%) | 6  (10.5%) |
| **Total** | 6436  (100%) | 3018  (100%) | 3361  (100%) | 57  (100%) |

## Table S7 – Bayesian multiple change point analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Male**  **systolic** | **Male**  **Diastolic** | **Female**  **Systolic** | **Female**  **diastolic** |
| **Intercept (mmHg)**  **(95% CI)**  **[Rhat]** | 21.1  (18.2 to 23.9) [1.033] | 33.4  (31.5 to 35.4) [1.007] | 18.4  (17.7 to 19.1) [1.023] | 25.1  (22.5 to 27.7)  [1.24] |
| **Slope (mmHg/year)**  **(95% CI)**  **[Rhat]** | -1.1  (-1.7 to -0.6)  [1.042] | -4.4  (-5 to -3.8)  [1.006] | -0.9  (-1 to -0.8)  [1.031] | -2.8  (-3.6 to -2.1)  [1.296] |
| **Change point age (years)**  **(95% CI)**  **[Rhat]** | 11.5  (7.8 to 17.6)  [1.039] | 5.9  (5.5 to 6.4)  [1.006] | 13.1  (12.2 to 14.1) [1.021] | 7.0  (5.9 to 8.2)  [1.274] |
| **Plateau (mmHg)**  **(95% CI)** | 9.2  (6.7 to 10.7)  [-] | 7.3  (6.8 to 7.9)  [-] | 6.4  (5.8 to 6.9)  [-] | 5.6  (4.8 to 6.5)  [-] |

Rhat is the Gelman-Rubin convergence statistic assessing the strength of Markov chain Monte Carlo model fits, values closer to 1.0 showing a stronger model estimate. Plateau is calculated from estimated changepoint, slope and intercept, and therefore does not have a calculated Rhat.

## Table S8 – Comprehensive Bayesian Joint Modelling parameters

### Model 1: Estimation of drug dose effects on BP

**Number of patients in model:** 452 **Number of visits in model:** 4187

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 102.047 | 96.551 to 107.542 | \* |
| **Sex (Male)** | 1.440 | -0.124 to 3.005 | NS |
| **Sex (Not assigned)** | -7.643 | -16.714 to 1.427 | NS |
| **Age (years)** | 0.084 | -0.428 to 0.595 | NS |
| **Height (cm)** | -0.083 | -0.156 to -0.009 | \* |
| **Weight (kg)** | 0.370 | 0.220 to 0.521 | \* |
| **Daily fludrocortisone (µg)** | 0.012 | 0.005 to 0.020 | \* |
| **Daily hydrocortisone equivalent (mg)** | 0.052 | -0.057 to 0.162 | NS |
| **Daily salt (g)** | -0.686 | -2.138 to 0.766 | NS |
| **SD of random slope | random intercept :**  0.80 | 4.97 | | **R2 systolic BP (95% CI) :**  0.27 (0.14 to 0.40) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 64.564 | 60.633 to 68.495 | \* |
| **Sex (Male)** | 0.713 | -0.394 to 1.820 | NS |
| **Sex (Not assigned)** | -3.061 | -9.261 to 3.138 | NS |
| **Age (years)** | 0.069 | -0.288 to 0.425 | NS |
| **Height (cm)** | -0.069 | -0.118 to -0.019 | \* |
| **Weight (kg)** | 0.184 | 0.107 to 0.262 | \* |
| **Daily fludrocortisone (µg)** | 0.008 | 0.003 to 0.014 | \* |
| **Daily hydrocortisone equivalent (mg)** | 0.031 | -0.045 to 0.107 | NS |
| **Daily salt (g)** | -0.562 | -1.540 to 0.416 | NS |
| **SD of random slope | random intercept :**  0.23 | 2.33 | | **R2 diastolic BP (95% CI) :**  0.17 (0.06 to 0.28) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

### Model 1: Sensitivity analysis A: Complete case analysis: Estimation of drug dose effect on BP

**Number of patients in model:** 370 **Number of visits in model:** 2204

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 98.682 | 90.895 to 106.468 | \* |
| **Sex (Male)** | 0.998 | -0.544 to 2.541 | NS |
| **Sex (Not assigned)** | -9.694 | -13.366 to -6.021 | \* |
| **Age (years)** | 0.068 | -0.473 to 0.609 | NS |
| **Height (cm)** | -0.080 | -0.158 to -0.002 | \* |
| **Weight (kg)** | 0.410 | 0.222 to 0.599 | \* |
| **Daily fludrocortisone (µg)** | 0.022 | 0.013 to 0.031 | \* |
| **Daily hydrocortisone equivalent (mg)** | 0.079 | -0.056 to 0.215 | NS |
| **Daily salt (g)** | -1.655 | -4.258 to 0.949 | NS |
| **SD of random slope | random intercept :**  1.12 | 7.40 | | **R2 systolic BP (95% CI) :**  0.27 (0.21 to 0.32) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 62.648 | 56.310 to 68.985 | \* |
| **Sex (Male)** | 0.688 | -0.452 to 1.828 | NS |
| **Sex (Not assigned)** | -2.778 | -6.878 to 1.322 | NS |
| **Age (years)** | 0.069 | -0.376 to 0.514 | NS |
| **Height (cm)** | -0.059 | -0.132 to 0.013 | NS |
| **Weight (kg)** | 0.179 | 0.123 to 0.234 | \* |
| **Daily fludrocortisone (µg)** | 0.014 | 0.006 to 0.021 | \* |
| **Daily hydrocortisone equivalent (mg)** | 0.061 | -0.025 to 0.147 | NS |
| **Daily salt (g)** | -1.389 | -2.888 to 0.110 | NS |
| **SD of random\_slope | random\_intercept :**  0.35 | 3.44 | | **R2 diastolic BP (95% CI) :**  0.17 (0.10 to 0.24) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications on complete data.

Conclusion of sensitivity analysis: imputation strategy is robust and leads to estimates of similar direction and magnitude as complete case analysis.

### Model 1: Sensitivity analysis B: Estimation of drug dose by BSA effect on BP SDS

**Number of patients in model:** 422 **Number of visits in model:** 3223

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence Interval** | **Significance** |
| **Outcome: Systolic BP (SD Score)** | | | |
| **Intercept** | 1.428 | 1.162 to 1.694 | \* |
| **Sex (Male)** | 0.163 | -0.007 to 0.334 | NS |
| **Age (years)** | -0.068 | -0.093 to -0.044 | \* |
| **Daily fludrocortisone per BSA**  **(µg/m2)** | 0.001 | 0.001 to 0.002 | \* |
| **Daily hydrocortisone equivalent per BSA (mg/m2)** | 0.003 | -0.010 to 0.016 | NS |
| **Daily salt per BSA**  **(g/m2)** | -0.021 | -0.116 to 0.073 | NS |
| **BMI SD Score** | 0.081 | 0.021 to 0.141 | \* |
| **SD of random slope | random intercept :**  0.08 | 0.54 | | **R2 systolic BP (95% CI) :**  0.31 (0.18 to 0.44) | |
| **Outcome: Diastolic BP (SD Score)** | | | |
| **Intercept** | 1.755 | 1.545 to 1.965 | \* |
| **Sex (Male)** | 0.163 | 0.019 to 0.307 | \* |
| **Age (years)** | -0.140 | -0.161 to -0.119 | \* |
| **Daily fludrocortisone per BSA (µg/m2)** | 0.001 | 0.001 to 0.002 | \* |
| **Daily hydrocortisone equivalent per BSA (mg/m2)** | 0.005 | -0.005 to 0.015 | NS |
| **Daily salt per BSA**  **(g/m2)** | 0.023 | -0.045 to 0.092 | NS |
| **BMI SD Score** | -0.007 | -0.052 to 0.037 | NS |
| **SD of random slope | random intercept :**  0.08 | 0.38 | | **R2 diastolic BP (95% CI) :**  0.42 (0.33 to 0.51) | |

BP = Blood pressure; SD = Standard deviation; BSA = Body surface area (calculated by the mosteller formula); BMI = body mass index; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

\*National Heart, Lung and Blood Institute data for calculation of SD scores of BP only available for those over 1 year, and not calculated for those patients declared sex ‘not assigned’ at birth, thus less data available for modelling.

Conclusion of sensitivity analysis: modelling of drugs by surface area and SD scores instead of absolute values does not make a significant difference to the direction or magnitude of the effect size of drugs on BP

### Model 1: Sensitivity analysis C: Estimation of drug dose effect on BP restricted to patients under 5 years of age

**Number of patients in model:** 381 **Number of visits in model:** 2895

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 85.87 | 74.96 to 96.78 | \* |
| **Sex (Male)** | 1.80 | -0.36 to 3.96 | NS |
| **Sex (Not assigned)** | -7.97 | -20.62 to 4.68 | NS |
| **Age (years)** | -1.66 | -3.10 to -0.22 | \* |
| **Height (cm)** | 0.25 | 0.04 to 0.45 | \* |
| **Weight (kg)** | -0.27 | -0.88 to 0.35 | NS |
| **Daily fludrocortisone (µg)** | 0.01 | 0.01 to 0.02 | \* |
| **Daily hydrocortisone equivalent (mg)** | 0.02 | -0.13 to 0.17 | NS |
| **Daily salt (g)** | -0.71 | -2.18 to 0.77 | NS |
| **SD of random slope | random intercept :**  0.63 | 4.47 | | **R2 systolic BP (95% CI) :**  0.20 (0.05 to 0.35) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 46.85 | 38.96 to 54.74 | \* |
| **Sex (Male)** | 0.50 | -1.05 to 2.05 | NS |
| **Sex (Not assigned)** | -2.79 | -13.03 to 7.44 | NS |
| **Age (years)** | -1.90 | -3.09 to -0.72 | \* |
| **Height (cm)** | 0.29 | 0.15 to 0.44 | \* |
| **Weight (kg)** | -0.46 | -0.91 to -0.00 | \* |
| **Daily fludrocortisone (µg)** | 0.01 | 0.00 to 0.02 | \* |
| **Daily hydrocortisone equivalent (mg)** | -0.01 | -0.13 to 0.12 | NS |
| **Daily salt (g)** | -0.50 | -1.45 to 0.45 | NS |
| **SD of random\_slope | random\_intercept :**  0.45 | 2.28 | | **R2 diastolic BP (95% CI) :**  0.14 (0.02 to 0.26) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications on complete data.

Conclusion of sensitivity analysis: Despite salt treatment being administered to only 15% of the sample and concentrated in those under 5, there is still no statistically significant effect on blood pressure of salt replacement when restricting analysis to patients under 5 years of age only.

### Model 2: Extent to which renin predicts BP

**Number of patients in model:** 544 **Number of visits in model:** 6130

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 108.85 | 103.06 to 114.63 | \* |
| **Sex (Male)** | 1.88 | 0.51 to 3.25 | \* |
| **Sex (Not assigned)** | -8.33 | -15.64 to -1.01 | \* |
| **Age (years)** | 0.33 | -0.07 to 0.73 | NS |
| **Height (cm)** | -0.15 | -0.22 to -0.07 | \* |
| **Weight (kg)** | 0.43 | 0.29 to 0.56 | \* |
| **ln renin (ln(µIU/ml))** | -1.00 | -1.47 to -0.53 | \* |
| **SD of random slope | random intercept :**  0.40 | 4.55 | | **R2 systolic BP (95% CI) :**  0.32 (0.19 to 0.45) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 68.7 | 64.20 to 73.20 | \* |
| **Sex (Male)** | 1.07 | 0.21 to 1.93 | \* |
| **Sex (Not assigned)** | -3.38 | -9.30 to 2.54 | NS |
| **Age (years)** | 0.29 | -0.07 to 0.66 | NS |
| **Height (cm)** | -0.10 | -0.16 to -0.05 | \* |
| **Weight (kg)** | 0.21 | 0.15 to 0.28 | \* |
| **ln renin (ln(µIU/ml))** | -0.71 | -1.13 to -0.29 | \* |
| **SD of random slope | random intercept :**  0.18 | 2.60 | | **R2 diastolic BP (95% CI) :**  0.21 (0.09 to 0.32) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

### Model 2: Sensitivity analysis A: Complete Case Analysis: Extent to which renin predicts BP

**Number of patients in model:** 256 **Number of visits in model:** 1027

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 99.74 | 88.17 to 111.30 | \* |
| **Sex (Male)** | 1.40 | -0.44 to 3.24 | NS |
| **Sex (Not assigned)** | -7.35 | -10.05 to -4.65 | \* |
| **Age (years)** | 0.17 | -0.66 to 1.01 | NS |
| **Height (cm)** | -0.05 | -0.20 to 0.09 | NS |
| **Weight (kg)** | 0.35 | 0.11 to 0.58 | \* |
| **ln renin (ln(µIU/ml))** | -1.13 | -1.71 to -0.54 | \* |
| **SD of random slope | random intercept :**  0.95 | 8.27 | | **R2 systolic BP (95% CI) :**  0.32 (0.26 to 0.39) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 61.28 | 54.33 to 68.24 | \* |
| **Sex (Male)** | 0.63 | -1.00 to 2.26 | NS |
| **Sex (Not assigned)** | -2.54 | -4.51 to -0.57 | \* |
| **Age (years)** | -0.02 | -0.66 to 0.61 | NS |
| **Height (cm)** | -0.02 | -0.10 to 0.07 | NS |
| **Weight (kg)** | 0.16 | 0.05 to 0.26 | \* |
| **ln renin (ln(µIU/ml))** | -0.80 | -1.20 to -0.41 | \* |
| **SD of random slope | random intercept :**  0.54 | 4.73 | | **R2 diastolic BP (95% CI) :**  0.24 (0.17 to 0.30) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) on complete data.

Conclusion of sensitivity analysis: imputation strategy is robust and leads to estimates of similar direction and magnitude as complete case analysis.

### Model 2: Sensitivity analysis B: Extent to which renin predicts BP SDS

**Number of patients: 499 Number of visits: 4564**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence Interval** | **Significance** |
| **Outcome: Systolic BP (SD Score\*)** | | | |
| **Intercept** | 1.89 | 1.75 to 2.03 | \* |
| **Sex (Male)** | 0.16 | -0.01 to 0.32 | NS |
| **Age (years)** | -0.09 | -0.11 to -0.07 | \* |
| **ln renin ln(µIU/ml)** | -0.08 | -0.12 to -0.04 | \* |
| **BMI SD Score** | 0.08 | 0.01 to 0.15 | \* |
| **SD of random slope | random intercept :**  0.08 | 0.50 | | **R2 systolic BP (95% CI) :**  0.33 (0.20 to 0.45) | |
| **Outcome: Diastolic BP (SD Score\*)** | | | |
| **Intercept** | 2.28 | 2.15 to 2.42 | \* |
| **Sex (Male)** | 0.18 | 0.05 to 0.30 | \* |
| **Age (years)** | -0.17 | -0.20 to -0.15 | \* |
| **ln renin ln(µIU/ml)** | -0.05 | -0.08 to -0.01 | \* |
| **BMI SD Score** | -0.02 | -0.07 to 0.02 | NS |
| **SD of random slope | random intercept :**  0.09 | 0.38 | | **R2 diastolic BP (95% CI) :**  0.42 (0.34 to 0.51) | |

BP = Blood pressure; SD = Standard deviation; BMI = body mass index calculated using world health organisation reference data; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

\*National Heart, Lung and Blood Institute data for calculation of SD scores of BP only available for those over 1 year, and not calculated for those patients declared sex ‘not assigned’ at birth, thus less data available for modelling.

Conclusion of sensitivity analysis: modelling of SD scores instead of absolute values does not make a significant difference to the direction or magnitude of the prediction of renin on BP

### Model 3: Extent to which 17OH-progesterone predicts BP

**Number of patients in model:** 544 **Number of visits in model:** 6130

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 106.64 | 101.58 to 111.69 | \* |
| **Sex (Male)** | 1.47 | 0.07 to 2.87 | \* |
| **Sex (Not assigned)** | -8.12 | -15.63 to -0.62 | \* |
| **Age (years)** | 0.18 | -0.20 to 0.55 | NS |
| **Height (cm)** | -0.12 | -0.18 to -0.05 | \* |
| **Weight (kg)** | 0.42 | 0.28 to 0.55 | \* |
| **ln 17OHP (ln(nmol/l))** | -0.64 | -1.00 to -0.27 | \* |
| **SD of random slope | random intercept :**  0.41 | 4.35 | | **R2 systolic BP (95% CI) :**  0.31 (0.17 to 0.45) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 67.37 | 63.72 to 71.02 | \* |
| **Sex (Male)** | 0.76 | -0.17 to 1.68 | NS |
| **Sex (Not assigned)** | -3.11 | -9.54 to 3.33 | NS |
| **Age (years)** | 0.18 | -0.16 to 0.51 | NS |
| **Height (cm)** | -0.08 | -0.13 to -0.03 | \* |
| **Weight (kg)** | 0.21 | 0.14 to 0.28 | \* |
| **ln 17OHP (ln(nmol/l))** | -0.50 | -0.78 to -0.22 | \* |
| **SD of random slope | random intercept :**  0.19 | 2.40 | | **R2 diastolic BP (95% CI) :**  0.20 (0.07 to 0.32) | |

BP = Blood pressure; SD = Standard deviation; 17OHP = 17OH-progesterone; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

### Model 3: Sensitivity analysis A: Complete Case Analysis: Extent to which 17OH-progesterone predicts BP

**Number of patients in model:** 288 **Number of visits in model:** 1215

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 94.52 | 84.07 to 104.97 | \* |
| **Sex (Male)** | 0.79 | -1.65 to 3.24 | NS |
| **Sex (Not assigned)** | -5.99 | -10.13 to -1.86 | \* |
| **Age (years)** | 0.07 | -0.63 to 0.77 | NS |
| **Height (cm)** | 0.02 | -0.12 to 0.15 | NS |
| **Weight (kg)** | 0.30 | 0.10 to 0.50 | \* |
| **ln 17OHP (ln(nmol/l))** | -0.86 | -1.26 to -0.46 | \* |
| **SD of random slope | random intercept :**  0.81 | 6.94 | | **R2 systolic BP (95% CI) :**  0.30 (0.24 to 0.37) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 60.14 | 54.60 to 65.69 | \* |
| **Sex (Male)** | 0.75 | -0.33 to 1.82 | NS |
| **Sex (Not assigned)** | -1.61 | -5.33 to 2.11 | NS |
| **Age (years)** | 0.14 | -0.40 to 0.68 | NS |
| **Height (cm)** | -0.01 | -0.07 to 0.06 | NS |
| **Weight (kg)** | 0.15 | 0.09 to 0.21 | \* |
| **ln 17OHP (ln(nmol/l))** | -0.64 | -0.88 to -0.40 | \* |
| **SD of random slope | random intercept :**  0.62 | 4.47 | | **R2 diastolic BP (95% CI) :**  0.22 (0.17 to 0.28) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) on complete data.

Conclusion of sensitivity analysis: imputation strategy is robust and leads to estimates of similar direction and magnitude as complete case analysis.

### Model 3: Sensitivity analysis B: Extent to which 17OH-progesterone predicts BP SDS

**Number of patients in model:** 499 **Number of visits in model:** 4564

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence Interval** | **Significance** |
| **Outcome: Systolic BP (SD Score\*)** | | | |
| **Intercept** | 1.89 | 1.75 to 2.03 | \* |
| **Sex (Male)** | 0.13 | -0.03 to 0.29 | NS |
| **Age (years)** | -0.09 | -0.11 to -0.07 | \* |
| **ln 17OHP (ln (nmol/l))** | -0.05 | -0.08 to -0.02 | \* |
| **BMI SD Score** | 0.08 | 0.01 to 0.15 | \* |
| **SD of random slope | random intercept :**  0.07 | 0.49 | | **R2 systolic BP (95% CI) :**  0.32 (0.19 to 0.45) | |
| **Outcome: Diastolic BP (SD Score\*)** | | | |
| **Intercept** | 2.3 | 2.17 to 2.43 | \* |
| **Sex (Male)** | 0.16 | 0.03 to 0.29 | \* |
| **Age (years)** | -0.17 | -0.20 to -0.15 | \* |
| **ln 17OHP (ln (nmol/l))** | -0.04 | -0.07 to -0.01 | \* |
| **BMI SD Score** | -0.02 | -0.07 to 0.02 | NS |
| **SD of random slope | random intercept :**  0.09 | 0.37 | | **R2 diastolic BP (95% CI) :**  0.42 (0.33 to 0.51) | |

BP = Blood pressure; SD = Standard deviation; BMI = body mass index calculated using world health organisation reference data; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

\*National Heart, Lung and Blood Institute data for calculation of SD scores of BP only available for those over 1 year, and not calculated for those patients declared sex ‘not assigned’ at birth, thus less data available for modelling.

Conclusion of sensitivity analysis: modelling of SD scores instead of absolute values does not make a significant difference to the direction or magnitude of the prediction of 17OHP on BP

### Model 3: Sensitivity analysis C: Complete Case Analysis: Extent to which 17OH-progesterone measured before 9am predicts BP

**Number of patients in model:** 66 **Number of visits in model:** 177

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 100.60 | 78.91 to 119.22 | \* |
| **Sex (Male)** | 4.12 | -0.75 to 8.88 | NS |
| **Sex (Not assigned)** | - | - | - |
| **Age (years)** | 0.06 | -2.05 to 3.05 | NS |
| **Height (cm)** | -0.12 | -0.39 to 0.14 | NS |
| **Weight (kg)** | 0.50 | 0.05 to 0.96 | \* |
| **ln 17OHP (ln(nmol/l))** | -0.13 | -0.87 to 0.65 | NS |
| **SD of random slope | random intercept :**  0.34 | 3.30 | | **R2 systolic BP (95% CI) :**  0.11 (0.06 to 0.18) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 68.38 | 49.79 to 86.46 | \* |
| **Sex (Male)** | 4.66 | 1.00 to 8.21 | NS |
| **Sex (Not assigned)** | - | - | - |
| **Age (years)** | 1.31 | -0.60 to 3.05 | NS |
| **Height (cm)** | -0.14 | -0.38 to 0.14 | NS |
| **Weight (kg)** | 0.02 | -0.37 to 0.42 | NS |
| **ln 17OHP (ln(nmol/l))** | -0.27 | -0.94 to 0.40 | NS |
| **SD of random slope | random intercept :**  0.36 | 1.41 | | **R2 diastolic BP (95% CI) :**  0.09 (0.04 to 0.15) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by Bayesian joint Model output using 400 iterations on complete data that also contained time of biomarker measurement. This had significant effect on sample size, reducing number of visits within this model of complete data from 1215 to 177.

Conclusion of sensitivity analysis: The larger confidence intervals and lack of statistical significance highlight there was insufficient data available with precise time of biomarker measurement to make any firm conclusions, but in combination with model 3 sensitivity analysis D this provides preliminary data to hypothesise that larger measurements of 17OHP later in the day may be more predictive of lower blood pressure due to poorer disease control than measurements of the same magnitude taken before 9am.

### Model 3: Sensitivity analysis D: Complete Case Analysis: Extent to which 17OH-progesterone measured at 9am or later in the day predicts BP

**Number of patients in model:** 92 **Number of visits in model:** 396

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 104.89 | 83.37 to 121.42 | \* |
| **Sex (Male)** | -1.79 | -5.68 to 2.24 | NS |
| **Sex (Not assigned)** | -6.44 | -18.43 to 7.19 | NS |
| **Age (years)** | -1.07 | -3.13 to 0.80 | NS |
| **Height (cm)** | -0.02 | -0.27 to 0.24 | NS |
| **Weight (kg)** | 0.56 | 0.11 to 0.97 | \* |
| **ln 17OHP (ln(nmol/l))** | -1.23 | -2.01 to -0.31 | \* |
| **SD of random slope | random intercept :**  0.30 | 2.21 | | **R2 systolic BP (95% CI) :**  0.10 (0.05 to 0.15) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 64.88 | 52.66 to 76.57 | \* |
| **Sex (Male)** | -0.28 | -2.79 to 2.34 | NS |
| **Sex (Not assigned)** | 0.53 | -9.13 to 9.39 | NS |
| **Age (years)** | -0.58 | -1.92 to 0.81 | NS |
| **Height (cm)** | 0.04 | -0.12 to 0.19 | NS |
| **Weight (kg)** | 0.08 | -0.25 to 0.38 | NS |
| **ln 17OHP (ln(nmol/l))** | -1.12 | -1.72 to -0.51 | \* |
| **SD of random slope | random intercept :**  0.20 | 1.77 | | **R2 diastolic BP (95% CI) :**  0.08 (0.04 to 0.13) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by Bayesian joint Model output using 400 iterations on complete data that also contained time of biomarker measurement. This had significant effect on sample size, reducing number of visits within this model of complete data from 1215 to 396.

Conclusion of sensitivity analysis: The larger confidence intervals and lack of statistical significance highlight there was insufficient data available with precise time of biomarker measurement to make any firm conclusions, but in combination with model 3 sensitivity analysis C this provides preliminary data to hypothesise that larger measurements of 17OHP later in the day may be more predictive of lower blood pressure due to poorer disease control than measurements of the same magnitude taken before 9am.

### Model 4: Extent to which Androstenedione predicts BP

**Number of patients in model:** 543 **Number of visits in model:** 6187

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 105.60 | 101.92 to 109.29 | \* |
| **Sex (Male)** | 1.30 | -0.35 to 2.95 | NS |
| **Sex (Not assigned)** | -9.42 | -19.72 to 0.87 | NS |
| **Age (years)** | 0.30 | -0.06 to 0.67 | NS |
| **Height (cm)** | -0.14 | -0.19 to -0.08 | \* |
| **Weight (kg)** | 0.44 | 0.35 to 0.54 | \* |
| **ln androstenedione (ln(nmol/l))** | -0.73 | -1.18 to -0.28 | \* |
| **SD of random slope | random intercept :**  0.41 | 4.38 | | **R2 systolic BP (95% CI) :**  0.31 (0.17 to 0.45) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 66.67 | 64.00 to 69.33 | \* |
| **Sex (Male)** | 0.76 | -0.57 to 2.09 | NS |
| **Sex (Not assigned)** | -4.20 | -12.85 to 4.46 | NS |
| **Age (years)** | 0.29 | 0.03 to 0.56 | \* |
| **Height (cm)** | -0.10 | -0.13 to -0.06 | \* |
| **Weight (kg)** | 0.22 | 0.16 to 0.28 | \* |
| **ln androstenedione (ln(nmol/l))** | -0.61 | -0.96 to -0.25 | \* |
| **SD of random slope | random intercept :**  0.20 | 2.39 | | **R2 diastolic BP (95% CI) :**  0.20 (0.08 to 0.32) | |

BP = Blood pressure; SD = Standard deviation; 17OHP = 17OH-progesterone; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

### Model 4: Sensitivity analysis A: Complete Case Analysis: Extent to which androstenedione predicts BP

**Number of patients in model:** 282 **Number of visits in model:** 1229

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence**  **Interval** | **Significance** |
| **Outcome: Systolic BP (Absolute values)** | | | |
| **Intercept** | 92.95 | 80.68 to 105.23 | \* |
| **Sex (Male)** | 0.87 | -0.60 to 2.34 | NS |
| **Sex (Not assigned)** | -10.83 | -24.93 to 3.27 | NS |
| **Age (years)** | 0.15 | -0.63 to 0.92 | NS |
| **Height (cm)** | -0.02 | -0.18 to 0.14 | NS |
| **Weight (kg)** | 0.38 | 0.27 to 0.48 | \* |
| **ln androstenedione (ln(nmol/l))** | -0.86 | -1.43 to -0.30 | \* |
| **SD of random slope | random intercept :**  0.91 | 7.73 | | **R2 systolic BP (95% CI) :**  0.32 (0.26 to 0.38) | |
| **Outcome: Diastolic BP (Absolute values)** | | | |
| **Intercept** | 61.74 | 55.96 to 67.53 | \* |
| **Sex (Male)** | 0.78 | -1.00 to 2.56 | NS |
| **Sex (Not assigned)** | -3.42 | -9.81 to 2.96 | NS |
| **Age (years)** | 0.12 | -0.24 to 0.47 | NS |
| **Height (cm)** | -0.05 | -0.13 to 0.03 | NS |
| **Weight (kg)** | 0.21 | 0.12 to 0.30 | \* |
| **ln androstenedione (ln(nmol/l))** | -0.72 | -0.95 to -0.49 | \* |
| **SD of random slope | random intercept :**  0.57 | 5.20 | | **R2 diastolic BP (95% CI) :**  0.25 (0.20 to 0.30) | |

BP = Blood pressure; SD = Standard deviation; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) on complete data.

Conclusion of sensitivity analysis: imputation strategy is robust and leads to estimates of similar direction and magnitude as complete case analysis.

### Model 4: Sensitivity analysis B: Extent to which androstenedione predicts BP SDS

**Number of patients in model:** 499 **Number of visits in model:** 4564

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Estimate** | **Confidence Interval** | **Significance** |
| **Outcome: Systolic BP (SD Score\*)** | | | |
| **Intercept** | 1.78 | 0.73 to 2.84 | \* |
| **Sex (Male)** | 0.12 | -0.04 to 0.28 | NS |
| **Age (years)** | -0.10 | -0.28 to 0.08 | NS |
| **ln androstenedione (ln(nmol/l))** | -0.06 | -0.25 to 0.13 | NS |
| **BMI SD Score** | 0.08 | 0.01 to 0.16 | \* |
| **SD of random slope | random intercept :**  0.07 | 0.49 | | **R2 systolic BP (95% CI) :**  0.32 (0.18 to 0.45) | |
| **Outcome: Diastolic BP (SD Score\*)** | | | |
| **Intercept** | 2.21 | 1.68 to 2.74 | \* |
| **Sex (Male)** | 0.15 | 0.02 to 0.28 | \* |
| **Age (years)** | -0.18 | -0.27 to -0.08 | \* |
| **ln androstenedione (ln(nmol/l))** | -0.04 | -0.19 to 0.10 | NS |
| **BMI SD Score** | -0.02 | -0.07 to 0.02 | NS |
| **SD of random slope | random intercept :**  0.09 | 0.37 | | **R2 diastolic BP (95% CI) :**  0.42 (0.33 to 0.51) | |

BP = Blood pressure; SD = Standard deviation; BMI = body mass index calculated using world health organisation reference data; CI = Confidence interval; NS = Not significant; \* = CI does not span zero. CI calculated by iterating Bayesian Model using 400 iterations each through 10 bootstrap replications (sampling all data available from one patient with replacement) for each of the 10 imputed datasets.

\*National Heart, Lung and Blood Institute data for calculation of SD scores of BP only available for those over 1 year, and not calculated for those patients declared sex ‘not assigned’ at birth, thus less data available for modelling.

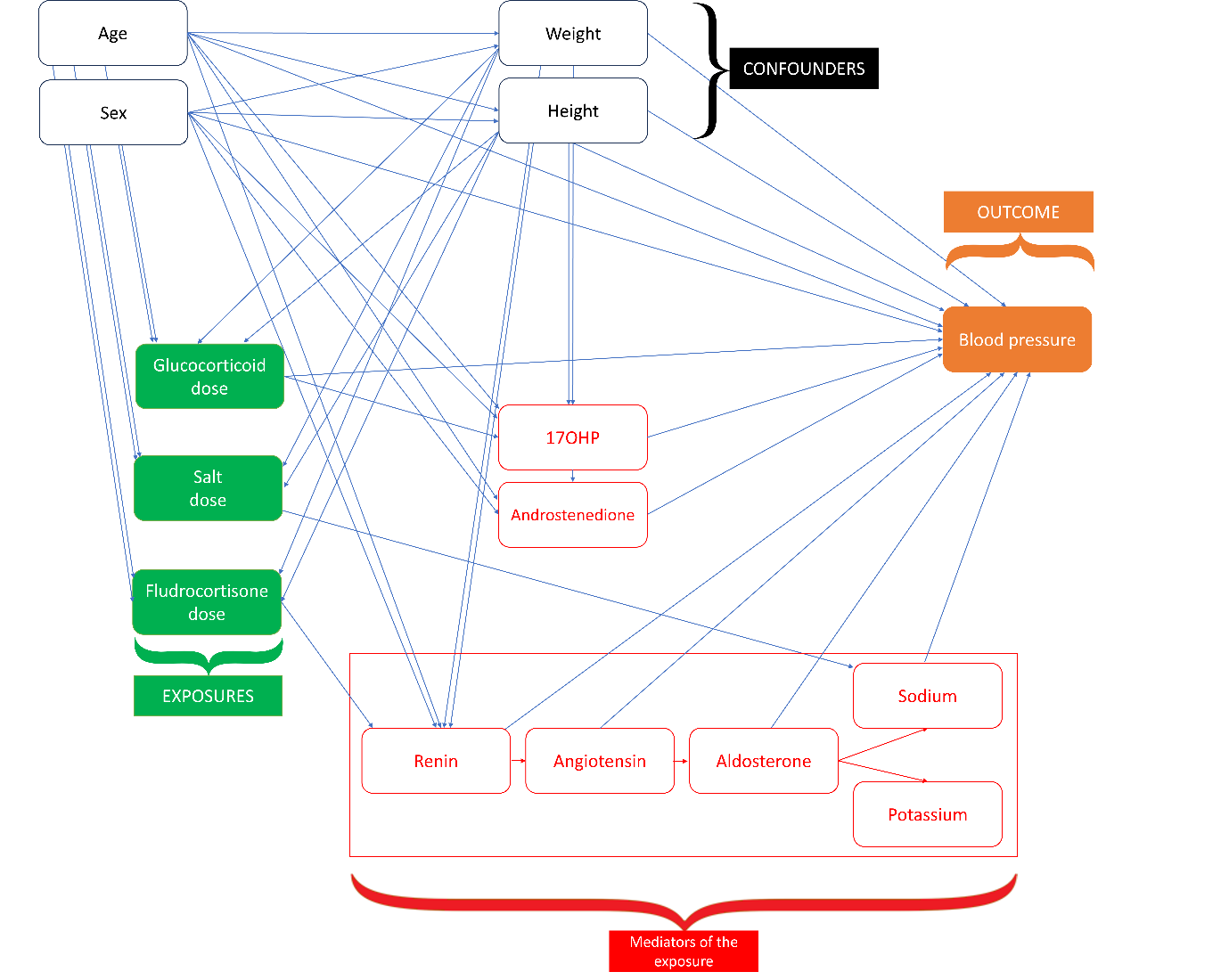
Conclusion of sensitivity analysis: modelling of SD scores instead of absolute values does not make a significant difference to the direction or magnitude of the prediction of androstenedione on BP

## Table S9 – Sensitivity analysis results: impact of imputation strategy on sample size and estimates

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model number: Target of estimation** | **Sample size with imputation (Patients**| **Visits)** | **Sample size without imputation (Patients**| **Visits)** | **Estimate with imputation (systolic)** | **Estimate without imputed data (systolic** | **Estimate with imputation (diastolic)** | **Estimate without imputed data (diastolic)** |
| **1: Effect of fludrocortisone on BP** | 452 | 4187 | 370 | 2204 | 0.012  (0.005 to 0.020) | 0.022  (0.013 to 0.031) | 0.008  (0.003 to 0.014) | 0.014  (0.006 to 0.021) |
| **2: Degree to which renin predicts BP** | 544 | 6130 | 256 | 1027 | -1.00  (-1.47 to -0.53) | -1.13  (-1.71 to -0.54) | -0.71  (-1.13 to -0.29) | -0.80  (-1.20 to -0.41) |
| **3: Degree to which 17OHP predicts BP** | 544 | 6130 | 288 | 1215 | -0.64  (-1.00 to -0.27) | -0.86  (-1.26 to -0.46) | -0.50  (-0.78 to -0.22) | -0.64  (-0.88 to -0.40) |
| **4: degree to which androstenedione predicts BP** | 543 | 6187 | 282 | 1229 | -0.73  (-1.18 to -0.28) | -0.86  (-1.43 to -0.30) | -0.61  (-0.96 to -0.25) | -0.72  (-0.95 to -0.49) |

BP=Blood Pressure. The sign and magnitude of all the estimates of interest are similar, and show a robust data imputation strategy and a high level of confidence in the effect sizes.

## Figure S1 – Directed acyclic graph



Relationship between variables of interest agreed through expert consensus. Arrow depicts direction of effect, but does not define the size or sign of the effect. Constructed to inform which confounding variables should be controlled for in analysis, whilst avoiding mediating variables or ancestors of variables that would introduce bias when interpreting regression coefficients. e.g. age is a covariate affecting BP and the dose of medication a patient is prescribed, and should thus be controlled for when estimating the effect of dose on BP. However, the effect of salt treatment will be mediated by increasing a patients’ sodium, and thus sodium should not be controlled for when assessing the effect of salt on BP. Appropriate covariate adjustment set for each target of estimation reported in table 3.

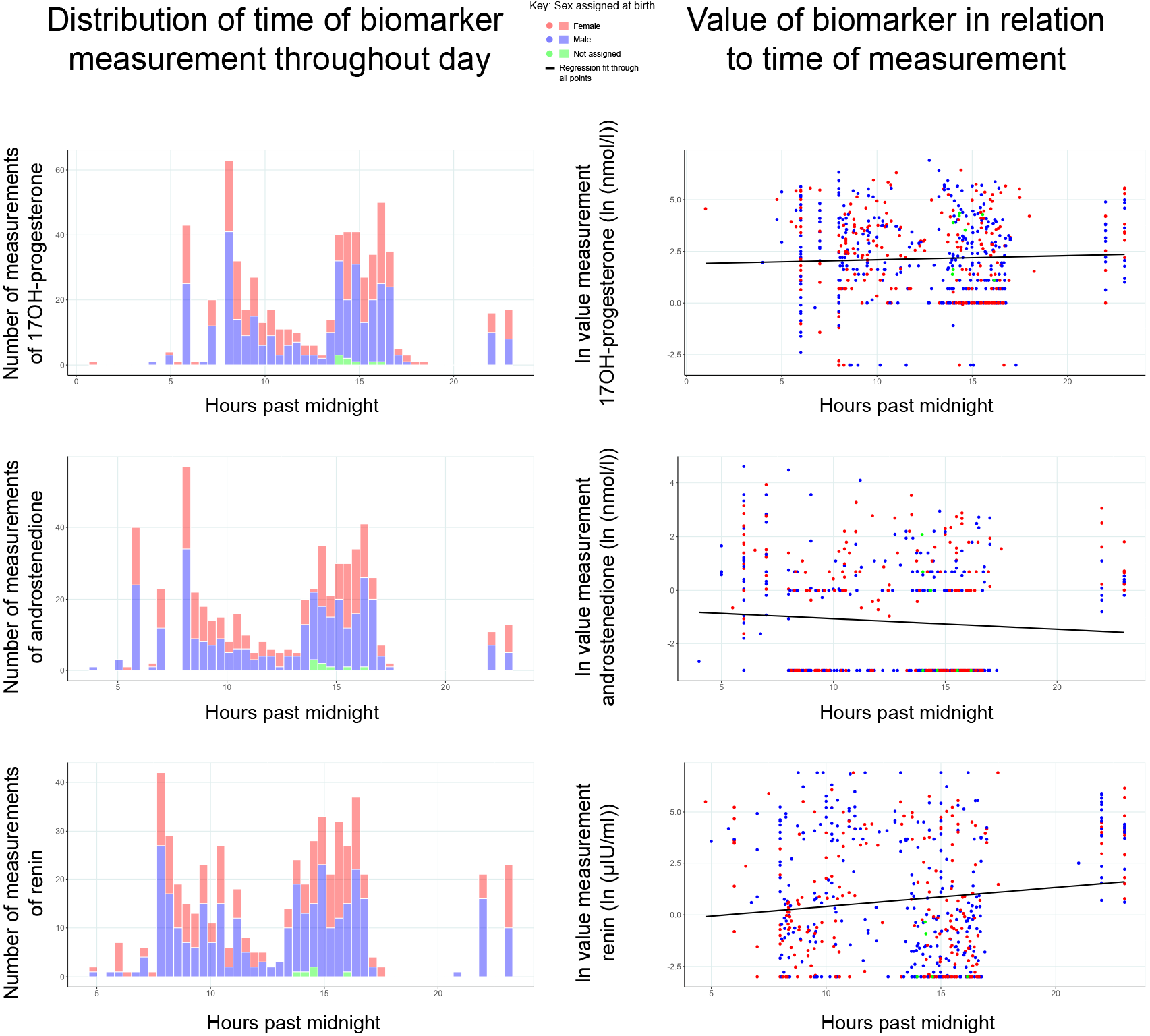
## Figure S2 – Histograms and regression of variables within models

\*Registry data contains dose prescribed from that clinic visit. To model the biometrics measured at a clinic visit, the dose the patient was prescribed at the previous visit is used. Height and weight are interpolated between points, but not extrapolated prior to the first data point available or beyond the last data point available. Dose data was imputed using last observation carried forward or next observation carried backward, but restricted to carrying for 6 months under age 1, 12 months under age 3, 24 months under age 5 and 36 months over age 5. Joint modelling multiple imputation of biomarkers and blood pressure only employed to visits with known or interpolated height and weight. Imputation therefore increases the amount available for modelling, but does not facilitate modelling across the entire dataset because of the pragmatic restrictions imposed to ensure the imputation strategy produced plausible data. Right hand column shows 1 imputation set as an example, but please note imputation was carried out 10 times with estimates calculated for each imputation and then collated using Rubin’s rules.

A group of graphs showing different colored dots

Description automatically generated with medium confidence

## Figure S3 – Distribution of biomarker measurements throughout the day



These plots show only biomarker measurements that had precise time of measurement recorded in the registry (<50% of values). See Table S5 for details of data for each biomarker that was missing a precise time of measurement within the registry.

## Figure S4 – Bayesian multiple change point analysis of difference between CAH patient blood pressure and normative values with age

Difference between median normative healthy child blood pressure values subtracted from median LMS modelled blood pressure of CAH (Congenital Adrenal Hyperplasia) patients with age. CAH patients have a larger difference in blood pressure at earlier ages. Lines show Bayesian multiple change point analysis that estimates the age where this difference stops decreasing to a plateau. Analysis shows the difference in diastolic blood pressure reduces at a faster rate than the difference in systolic blood pressure for both sexes. indicating ages at which the difference between CAH patients and normative values stops decreasing. Plateau of each line shows the amount CAH patients have greater blood pressure than normative values for each reading.