CHIPS-Child: Testing the developmental programming hypothesis in the offspring of the CHIPS trial

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ABSTRACT

Objectives: As a follow-up to the CHIPS trial (Control of Hypertension In Pregnancy Study) of 'less tight' (versus 'tight') control of maternal blood pressure in pregnancy, CHIPS-Child investigated potential developmental programming of maternal blood pressure control in pregnancy, by examining measures of postnatal growth rate and hypothalamic-pituitary adrenal (HPA) axis activation.

Methods: CHIPS follow-up was extended to 12 ± 2 months corrected post-gestational age for anthropometry (weight, length, head/waist circumference). For eligible children with consent for a study visit, we collected biological samples (hair/buccal samples) to evaluate HPA axis function (hair cortisol levels) and epigenetic change (DNA methylation analysis of buccal cells). The primary outcome was 'change in z-score for weight' between birth and 12 ± 2 mos. Secondary outcomes were hair cortisol and genome-wide DNA methylation status.

Results: Of 683 eligible babies, 183 (26.8%) were lost to follow-up, 83 (12.2%) declined, 3 (0.4%) agreed only to ongoing contact, and 414 (60.6%) consented. 372/414 (89.9%) had weight measured at 12mos. In 'less tight' (vs. 'tight') control, the primary outcome was similar [−0.26 (−0.53, +0.01); p = 0.14, p_adjusted = 0.06]; median (95% confidence interval) hair cortisol (N = 35 samples) was lower [−496 (−892, −100) ng/g;

p = 0.02], and buccal swab DNA methylation (N = 16 samples) was similar. No differences in growth rate could be demonstrated up to 5 years.

Conclusions: Results demonstrate no compelling evidence for developmental programming of growth or the HPA axis. Clinicians should look to the clinical findings of CHIPS to guide practice. Researchers should seek to replicate these findings and extend outcomes to paediatric blood pressure and neurodevelopment.

1. Introduction

The Developmental Origins of Health and Disease (DOHaD) concept refers to the process by which an exposure at a specific time during fetal or early infant development may have long-lasting or persistent effects on tissue structure or function after birth [1–3]. According to the DOHaD concept, the fetus will respond to prenatal cues and adapt to its environment, resulting in physiological changes to the phenotype of the fetus [3,4]. These changes are believed to facilitate survival of the child to reproductive age, known as Darwinian Fitness [5]. However, these changes can predispose a child to be mismatched to the environment it finds itself in as an adult, which can lead to the development of non-communicable diseases (NCD), such as cardiovascular disease and diabetes [5–7]. For example, there is strong evidence that fetal under-nutrition is associated with adult cardiovascular disease, and that the heightened risk is evident across the spectrum of normal birthweight [8–10].

Epigenetics (the study of how the epigenome – which regulates gene expression – is affected by environmental factors) is the current concept to explain how exposures during early human development may be linked with childhood or adult outcomes. Epigenetic changes have been shown to be reversible during a critical time frame of developmental plasticity [5]. However, these changes can also predispose cells to future malfunction and possible disease [11,12]. Epigenetic changes have been reported following exposures at specific points in fetal development (e.g., conception in vitro) [13] and after prolonged exposure during pregnancy (e.g., smoking, famine/nutrition, maternal overweight) [14–16]. Animal and human studies have shown that environmental effects (e.g., nutritional cues, maternal behaviour) on the fetal and newborn epigenome may be sustained as far as weaning and into early childhood [17,18,3], leading to descriptions of the epigenome as the 'archive' of the prenatal environment [19].

A 'lifecourse' approach to NCD prevention, from pre-conception, through pregnancy, early years of life, and beyond has been advocated by the United Nations and emphasised by the Sustainable Development Goals, known as 'SDGs' [3,5,6,11]. Public Health policies globally should consider human development as a crucial factor in diminishing the risk of NCDs in all societies. However, a major challenge for human studies of DOHaD is demonstration of exposure-outcome relationships. Although one can manipulate the maternal or fetal environment to some extent, clearly, it is neither ethical nor feasible in humans to intentionally deprive the mother or fetus of nutrition, or expose the mother or infant to prolonged stress. Yet, whether developmental programming occurs in response to interventions in pregnancy is of vital importance to maternity care providers. To date, human studies have taken epidemiological approaches (mostly retrospective and cross-sectional) and drawn inference (but not causation) from statistical associations of variables and outcomes [20].

The CHIPS Trial (Control of Hypertension In Pregnancy Study, NCT01192412) was a rigorous randomised controlled trial of BP control during pregnancy, designed to keep mothers safe and optimise outcomes for babies [21]. CHIPS tested the hypothesis that 'less tight' (vs. 'tight') BP control would improve fetal growth (which it may have) and through that, reduce the risk of death or illness for the baby (which it did not). This hypothesis was based on the premise that 'less tight' BP control affects maternal vasculature leading to improved nutrient delivery to the fetus. CHIPS recruited 987 women in 94 centres internationally. Women randomised to 'less tight' control (target diastolic BP 100 mmHg), compared with 'tight' control (target diastolic BP 85 mmHg) of maternal BP, had similar rates of serious complications for the baby or mother, while there was an increase in other risks for the mother (i.e., more severe maternal hypertension, low platelet counts, and elevated liver enzymes). Although in 'less tight' (vs. 'tight') control group, fewer babies may have been born with a birth weight < 10th centile' (not significant), an opposite trend of similar strength was seen in preterm birth (also not significant).

CHIPS-Child was a separate study that extended follow-up in CHIPS to examine potential developmental programming associated with maternal BP control, independently from genetic and environmental factors such as prenatal nutrition. Follow-up of the babies was extended to measure growth, hypothalamic-pituitary adrenal (HPA) axis function, and epigenetic change at age 12 months. CHIPS-Child tested the hypothesis that babies in the 'less tight' control group would have had: (i) better antenatal nutrition and a slower rate of postnatal growth compared with babies in the 'tight' control who may have exhibited accelerated postnatal 'catch-up' growth (our primary measure of developmental programming) in the postnatal period [10,12,22–25] ; (ii) less HPA axis activation; and (iii) changes in DNA methylation that may mediate these findings.

2. Methods

CHIPS sites that were interested in participating in CHIPS-Child extended CHIPS follow-up from six weeks postpartum until either September 2015 or when the child was five years of age, whichever was earlier. CHIPS-Child required specific ethics approval, in addition to CHIPS, at the central co-ordinating centre, University of British Columbia (Oct 13, 2011, H08-00882), and at each study site.

Site co-ordinators contacted (by phone, email, or in person at hospital visits) CHIPS participants who had: (i) experienced a live birth without neonatal death in CHIPS, and (ii) not withdrawn their consent or been lost to follow-up antepartum or postpartum during CHIPS. All participants gave fully informed written consent to participate in CHIPS-Child.

The type of potential CHIPS-Child participation depended on the age of the child at study contact and the interest of the parent/guardian, as follows.

1) When children were ≤14 months corrected post-gestational age at the time of study contact with the parent, s/he was approached for consent to 'FULL' participation of the child. This involved a face-to-face study visit for the child at 12 ± 2 months corrected post-gestational age. Anthropomorphic measurements were taken, biological samples collected, and a maternal questionnaire completed (see below). The anthropomorphic measurements were weight, length, and waist and head circumference (see below). Hair samples were obtained for evaluation of cortisol as a measure of HPA axis activation. Buccal swabs were taken for extraction of DNA and epigenetic testing by DNA methylation. (For details of the hair and buccal swab collection, see Appendix S1). The maternal questionnaire covered details of maternal education, post-randomisation practices not collected in CHIPS (such as exposure to environmental smoking), and infant feeding practices.

2) When children were ≥15 months at first study contact, or if the family declined FULL participation, the parent/guardian was approached for consent to 'LIMITED' participation of the child. This

involved giving consent for the site co-ordinator to obtain (from family doctors, paediatricians, or health clinics, as relevant) the child's weight and length measurements taken during routine clinical visits at 12 ± 2 months corrected post-gestational age. At 28 months into the study, a protocol amendment was made so that the parent/guardian was also asked for their consent to complete the maternal questionnaire by phone; those parents/guardians who had already consented to LIMITED participation had to re-consent prior to questionnaire completion. No biological samples were collected from LIMITED participants as they were not physically seen.

3) When the parent/guardian declined both FULL and LIMITED participation for their child, the parent/guardian was, as for all participants, approached for consent to 'maintenance of contact' every four months throughout the study period (i.e., Oct 2011–Sept 2015) or until the child turned 5 years of age. This maintenance of contact was designed to confirm contact details and leave open the option of further follow-up.

At the time of receipt of written consent to CHIPS-Child, all families had been sent a CHIPS-Child tape measure, wall-mounted height chart, and instructions for how to take each of the measurements, with particular attention paid to the correct placement of the wall chart on the wall, and tape measure on the child for measurement of waist circumference. Thus, on the approximate date of the child's birthday (corrected post gestational age ± 2 months), families were sent a stamped postcard with a request for the child's weight, height, and waist circumference measurements to be recorded and returned to the study co-ordinator.

Our primary outcome was 'change in z-score for weight' between birth and 12 months corrected post-gestational age, using the Fenton *et al.* 2013 calculator [26] for weight at birth (collected in CHIPS) and the World Health Organization (WHO) calculator [27] for weight at 12 ± 2 months. Our secondary outcomes were hair cortisol levels and genome-wide DNA methylation status. Other outcomes included between-group differences in other anthropometry measures at i.e., 12 ± 2 months and all anthropometry, annually from 2 to 5 years.

2.1. Statistics

At participating sites, comparability of our follow-up population was assessed by comparing maternal pre-randomisation [i.e., centre, type of hypertension, type of antihypertensive, and maternal body mass index (BMI)] and livebirth characteristics (i.e., gestational age at delivery, birth weight, and high level neonatal care for > 48 h) between children who were followed-up and those who were not. A similar analysis was then undertaken for children who were followed up, according to whether their mothers had been in 'less tight' vs. 'tight' control groups in CHIPS. Fisher's Exact, Student's t-, or Mann-Whitney U tests were used, as applicable, for categorical or continuous variables. A p value < 0.05 was considered statistically significant. The unit of analysis throughout was the woman (pregnancy). There were no multiple pregnancies in CHIPS.

The primary outcome was compared between 'less tight' and 'tight' control groups using linear regression, adjusted for maternal pre-randomisation factors (hypertension type and centre, antihypertensive type, BMI, and any between-group differences at baseline among babies followed-up) (two-tailed p < 0.05). In an exploratory analyses (because differences could have resulted, at least in part, by the intervention itself), adjustment was made for post-randomisation antenatal or postnatal influences on the baby's postnatal weight gain (most notably nutrition) that may have resulted in between-group differences: post-randomisation antenatal maternal smoking or intake of folate-containing vitamins, socioeconomic status (as reflected by country of origin and maternal years of education), livebirth characteristics (i.e.,
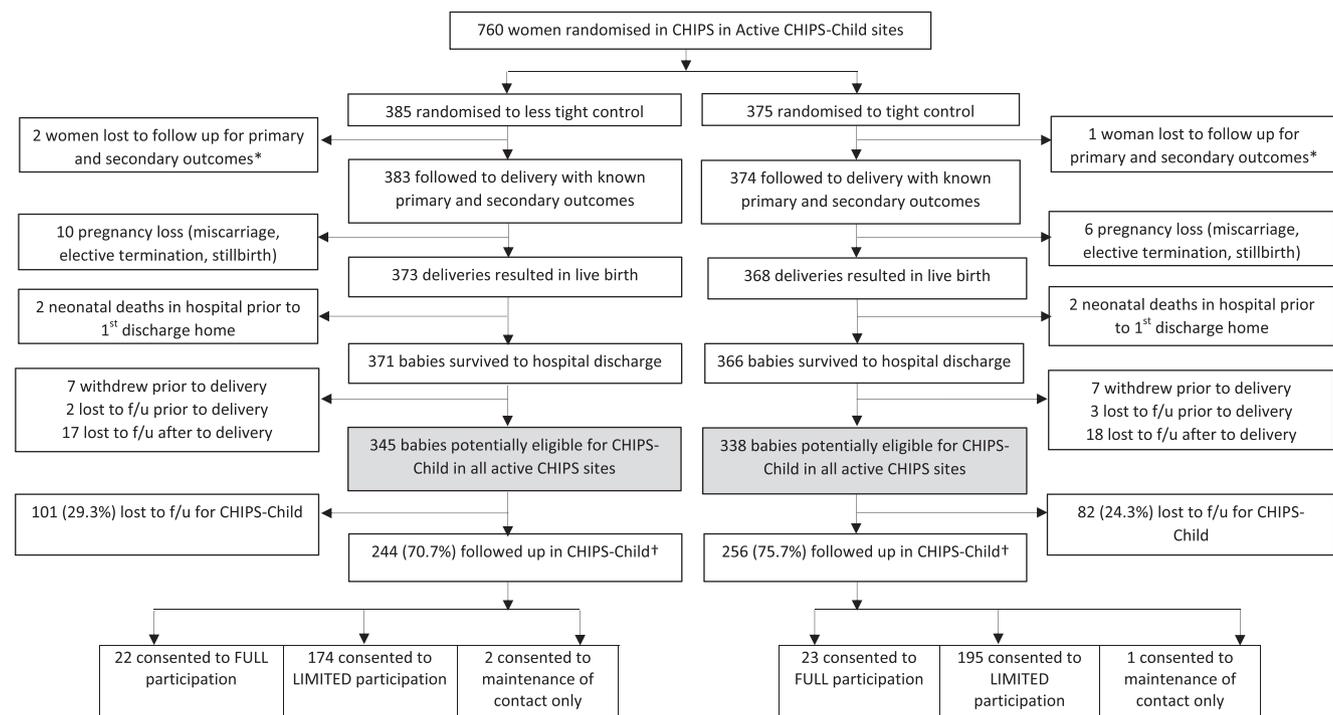
Fig. 1. CHIPS Trial and CHIPS-Child study profile (for details about "rows", see 'flow through CHIPS table below footnotes). NND (neonatal death), PPQ (postpartum questionnaire). * These are the women who are in the CHIPS Consort Diagram and for whom we have no primary and secondary outcome data. § There were 83 women who declined particpation, 46 in 'less tight' and 37 in 'tight'.

gestational age at delivery, birth weight, and high level neonatal care for > 48 h), and measures of early postnatal nutrition (i.e., breast-feeding to any extent, and the presence of health or dental problems that may have affected eating). With a two-tailed alpha of 0.05 and SD of the z-score of 1 (by definition), we calculated that we would have > 80% power to detect a clinically relevant between-group difference in 'change in z-score for weight' of 0.25 with follow-up as low as 253/group.

The rate of ongoing growth (in terms of weight), was assessed annually following an age of 12 ± 2 months, and compared between groups by mixed effects linear regression, with centre and subject as a random effect, and adjustment for maternal pre-randomisation factors listed above for analysis of the primary outcome. A mixed effects approach was taken to account for the inclusion of different children at different time points, based on their age at follow-up contact in CHIPS-Child, and the maximal age that they could reach when the study ended in September 2015 (which depended on whether they had been recruited earlier or later in the course of the CHIPS Trial, 2009-12). For the year 2–5 growth measurements, we included only babies whose growth measurements were taken by parents on or after recruitment to CHIPS-Child, as parents needed to receive the study materials in order to take accurate measurements.

Hair cortisol was measured using a salivary enzyme immunoassay kit. Hair cortisol, a continuous variable, was compared between groups using multiple linear (least-squares) regression analysis, adjusted for type of hypertension, maternal BMI, and duration of participation in CHIPS.

DNA methylation status (from buccal swab DNA) was assessed genome-wide, between 'less tight' and 'tight' control groups, using a false discovery rate (FDR) calculation based on 'Significance Analysis of Microarrays' [28]. This method assigns a score to each DNA site evaluated on the basis of change in measurement relative to the SD of repeated measurements. The technique is more appropriate to address the multiple-test problem present in microarray data than is using the Bonferroni correction. The FDR threshold is determined from the observed distribution of p-values (i.e., the number of results with a specific p is calculated relative to those expected by chance). Differentially methylated sites were prioritised based on: (i) a FDR < 10% (meaning that > 90% of sites identified as significant are likely true findings), (ii) a between-group difference in means > 10% (as a measure a biologically significant difference), and (iii) the site(s) having a potential role in growth/HPA axis activation. Any candidate-altered sites were to be verified using pyrosequencing.

'Other' outcomes were compared between groups using a two-sample *t*-test for continuous outcomes, and chi-square or Fisher's Exact test (as appropriate) for categorical outcomes.

3. Results

3.1. Participants

Fifty-nine of the 94 CHIPS sites (62.8%), with 760/987 CHIPS participants (77.0%), chose to participate in CHIPS-Child. Fig. 1 describes the flow of these women through CHIPS into CHIPS-Child, resulting in 683 babies who were potentially eligible for CHIPS-Child in 'less tight' (N = 345) and 'tight' control (N = 338) groups. 183 (101 vs. 82, 26.8% overall) CHIPS-Child participants were lost to follow-up, leaving 500 (244/345, 70.7% in 'less tight' and 256/338, 75.7% in 'tight' control, p = 0.14) who were successfully contacted and 414 (196/244, 80.3% in 'less tight' and 218/256, 85.2% in 'tight' control, p = 0.19) who consented to participation.

Table 1 shows that CHIPS participants who were followed-up, compared with those who were not, were more likely to be from high perinatal mortality ratio (PMR) countries, especially South America. However, most subjects still came from North America (32.9%) and the UK/Europe (44.1%).

The nature of potential study participation depended on the age of the child at first study contact which was almost always with the mother (240, 98.4% in 'less tight' and 253, 98.8% in 'tight'; p = 0.72) and at a median [IQR] corrected post-gestational age of 27.7 [18.0, 38.2] months in 'less tight' and 29.2 [20.4, 38.8] months in 'tight' (p = 0.49). At first contact, only 92 children (47, 19.3% in 'less tight' and 45, 17.6% in 'tight') were actually eligible for a study visit based on a corrected post-gestational age of < 15 months; 81 children, plus one additional child who was actually ≥15 months at the time of contact, were invited to undertake FULL participation, of whom 45 (54.9%) consented (22/41, 53.7% in 'less tight' and 23/41, 56.1% in 'tight'). Those who did not consent to FULL participation, as well as the 407 other children who were ≥15 months at the time of first study contact, were all approached to undertake LIMITED participation; 369/455 (81.1%) consented. The remaining 3 participants (2 in 'less tight' and 1 in 'tight') consented only to maintenance of contact. Fig. 1 summarises

Table 1
Comparability of participants followed to those not followed-up at CHIPS-Child participating sites.

Variable	All CHIPS Child sites (n = 683)	Women NOT followed (n = 183)	Women followed (n = 500)	P*
Maternal pre-randomisation factors				
PMR of recruiting country				0.020
Low (< 10/1000 births)	602 (88.1)	170 (92.9)	432 (86.4)	
High (≥10/100 births)	81 (11.9)	13 (7.1)	68 (13.6)	
Regions of recruiting sites				0.028
Australasia	67 (9.8)	17 (9.3)	50 (10.0)	
Middle East	22 (3.2)	2 (1.1)	20 (4.0)	
North America	225 (32.9)	74 (40.4)	151 (30.2)	
South America	68 (10.0)	12 (6.6)	56 (11.2)	
UK and Europe	301 (44.1)	78 (42.6)	223 (44.6)	
Type non-proteinuric hypertension				0.538
Pre-existing hypertension	508 (74.4)	133 (72.7)	375 (75.0)	
Gestational hypertension	175 (25.6)	50 (27.3)	125 (25.0)	
Antihypertensive therapy				
Use at randomisation	370 (54.2)	101 (55.2)	269 (53.8)	0.747
Type at randomisation				0.053
Labetalol ± other (not methyldopa)	152 (22.3)	53 (29.0)	99 (19.8)	
Methyldopa ± other (not labetalol)	161 (23.6)	35 (19.1)	126 (25.2)	
Other	57 (8.3)	13 (7.1)	44 (8.8)	
None	313 (45.8)	82 (44.8)	231 (46.2)	
BMI (kg/m²)				0.138
< 25	161 (23.8)	36 (19.8)	125 (25.3)	
≥25	516 (76.2)	146 (80.2)	370 (74.7)	
Unknown	6	1	5	
(kg/m²)	31.2 (7.8)	31.7 (7.7)	31.0 (7.8)	0.264
Live birth characteristics (post-randomisation)†				
Gestational age at delivery (wks)	37.3 (2.8)	37.4 (2.7)	37.3 (2.8)	0.509
Delivery at < 37 wks	201 (29.4)	52 (28.4)	149 (29.8)	0.725
Delivery at < 34 wks	78 (11.4)	19 (10.4)	59 (11.8)	0.606
Birth weight (g)	2909.3 (820)	2875.8 (760.6)	2921.6 (841.1)	0.518
< 2500 g	176 (25.8)	46 (25.1)	130 (26.0)	0.819
< 1250 g	28 (4.1)	6 (3.3)	22 (4.4)	0.513
< 10th centile	115 (16.8)	33 (18.0)	82 (16.4)	0.614
< 3rd centile	26 (3.8)	5 (2.7)	21 (4.2)	0.375
High level neonatal care for > 48 h	194 (28.4)	48 (26.2)	146 (29.2)	0.446

(N (%) and mean ± SD unless otherwise stated), *BMI (body mass index), PMR (perinatal mortality ratio)*.

* The groups were compared using the Student's *t*-test.

† These characteristics were used for adjustment only in exploratory analyses.

the nature of participation among the 500 participants who were followed up: 83 (16.6%; 46, 18.9% in 'less tight' and 37, 14.5% in 'tight') did not consent to participate in any way; 45 (9.0%; 22, 9.0% in 'less tight' and 23, 9.0% in 'tight') consented to FULL participation; 369 (73.8%; 174, 71.3% in 'less tight' and 195, 76.2% in 'tight') to LIMITED participation; 3 (0.6%; 2, 0.8% in 'less tight' and 1, 0.4% in 'tight') consented to maintenance of contact only.

Table 2 shows that of CHIPS-Child participants, babies in 'less tight' (vs. 'tight') control were more likely to be born at an earlier gestational age, similar to what we reported in CHIPS [13].

3.2. Postnatal growth

Of 414 CHIPS-Child participants who consented to FULL or LIMITED participation, the infant's weight was recorded for 372 (89.9%) (175/196, 89.3% in 'less tight' and 197/218, 90.0% in 'tight'), either by the study co-ordinator at the study visit (N = 45, 22 in 'less tight' and 23 in 'tight') or by doctors in their offices as part of routine care (N = 327, 153 in 'less tight' and 174 in 'tight'). Of these 372 measurements, 294 (79.0%) (137, 78.3% in 'less tight' and 157, 79.7% in 'tight') were within the 12 ± 2 months specified in the primary outcome. Supplementary Table S2 shows that there was no difference in pre-randomisation maternal (or post-randomisation live birth characteristics) between children in 'less tight' vs. 'tight' control who had weight recorded at 12 months (vs. those who did not).

The CHIPS-Child primary outcome, change in z-score for weight between birth and 12 ± 2 months, appeared to be lower in 'less tight' (vs. 'tight') control, but the result was not statistically significant in analyses that were unadjusted (−0.21, 95% CI −0.48, +0.07; p = 0.14) or adjusted for maternal pre-randomisation factors (see methods) (−0.26, 95% CI −0.53, +0.01; p = 0.06). In the exploratory analysis that also adjusted for post-randomisation antenatal and postnatal influences on postnatal weight gain (see methods), a between-group difference in 'change in z-score for weight' was not evident (−0.08, 95% CI −0.34, +0.18; p = 0.54). These adjustments included infant feeding practices for which there were no obvious differences between groups. Those practices were: breastfeeding at any point (117, 83.4% in 'less tight' vs. 121, 84.0% in 'tight'), a perception that the infant was not eating adequately (mean score of 7.1 ± 2.1 in 'less tight' and 7.3 ± 2.0 in 'tight' on a self-rated scale of 1–10, with 1 being 'poor food intake' and 10 being 'excellent'), or the presence of any medical or dental problems that could affect eating (15, 10.7% in 'less tight' vs. 16, 11.1% in 'tight').

There was no significant difference in annual postnatal growth rate from birth to 5 years in 'less tight' (vs. 'tight') control, despite the impression that postnatal growth rate may be slower until two years and then more rapid from three years (Fig. 2, Supplementary Table S3). Similar results were seen when growth was assessed relative to birth (Supplementary Table S4). The number of babies with annual weight measurements decreased over time: 129/180 (71.7%) at 2 years, 147/300 (49.0%) at 3 years, 83/204 (40.7%) at 4 years, and 21/70 (30.0%) at 5 years of age. Any apparent reduction in follow-up in the 'less tight' (vs. 'tight') control over time did not reach statistical significance at 2 years (65/91, 71.4% vs. 64/89, 71.9%; p = 0.94), 3 years (71/145, 49.0% vs. 79/155, 51.0%; p = 0.73), 4 years (32/92, 34.8% vs. 54/112, 48.2%; p = 0.05), or 5 years (5/29, 17.2% vs. 16/41, 39.0%; p = 0.05) although data were limited at this last time point. These numbers reflected attrition of some children and the addition of others who had their anthropometry taken by parents after CHIPS-Child recruitment; by September 2015 when the study ended, all children had turned 2 years of age (although N = 133 were not recruited prior to this birthday), most (N = 187) had turned 3 years, many had reached 4 years (N = 132) and some had reached 5 years of age (N = 59).

For the 'other' anthropometry outcomes of length (at 12 ± 2 mos), standing height (at 2–5 years), head circumference (at 12 ± 2 mos), and waist circumference (at years 1–5), there were no differences

between 'less tight' and 'tight' control groups (Supplementary Table S5).

3.3. Secondary outcomes: hair cortisol and DNA methylation

Secondary outcomes were examined for the 45 children who attended a study visit as part of FULL participation. Hair samples were collected from 41 children (91.1%) (18/22 in 'less tight' and 23/23 in 'tight' control). Four children in 'less tight' control did not have hair collected because the child did not have enough hair to collect, or the parent considered it culturally unacceptable to cut the hair, s/he did not want the sample sent overseas, or s/he did not want their child to participate in this part of the study (one each). 35/41 (85.4%, 15 in 'less tight' and 20 in 'tight') hair samples contained sufficient hair to measure cortisol. The characteristics of these children were not statistically different between groups (Supplementary Table S6).

Median [IQR] hair cortisol was significantly lower in 'less tight' (vs. 'tight') control (261.7 [33.5, 519.8] vs. 668.8 [316.4, 1039.9] ng/g; p = 0.03), a difference that persisted following adjustment (see methods) (−495.82 [−891.62, −100.01]; p = 0.02). The difference

Table 2
Comparability of CHIPS-Child groups according to allocated treatment in CHIPS.

Variable	'Less tight' control (n = 244)	'Tight' control (n = 256)	P[*]
Maternal pre-randomisation factors			
PMR of recruiting country			0.757
Low (< 10/1000 births)	212 (86.9)	220 (85.9)	
High (≥10/100 births)	32 (13.1)	36 (14.1)	
Region of recruiting sites			0.884
Australasia	25 (10.2)	25 (9.8)	
Middle East	8 (3.3)	12 (4.7)	
North America	71 (29.1)	80 (31.3)	
South America	27 (11.1)	29 (11.3)	
UK and Europe	113 (46.3)	110 (43.0)	
Type non-proteinuric hypertension			0.679
Pre-existing hypertension	185 (75.8)	190 (74.2)	
Gestational hypertension	59 (24.2)	66 (25.8)	
Antihypertensive therapy			
Use at randomisation	127 (52.0)	142 (55.5)	0.443
Type at randomisation			0.684
Labetalol ± other (not methyldopa)	43 (17.6)	56 (21.9)	
Methyldopa ± other (not labetalol)	62 (25.4)	64 (25.0)	
Other	22 (9.0)	22 (8.6)	
None	117 (48.0)	114 (44.5)	
BMI (kg/m²)			0.696
< 25	63 (26.0)	62 (24.5)	
≥25e	179 (74.0)	191 (75.5)	
Unknown	2	3	
(kg/m²)	30.9 (7.8)	31.0 (7.8)	0.919
Live birth characteristics (post-randomisation)[†]			
Gestational age at delivery (wks)	37.0 (3.0)	37.6 (2.6)	0.019
Delivery at < 37 wks	82 (33.6)	67 (26.2)	0.069
Delivery at < 34 wks	34 (13.9)	25 (9.8)	0.149
Birth weight (g)	2893.7 (869.3)	2948.5 (814.1)	0.469
< 2500 g	65 (26.6)	65 (25.4)	0.750
< 1250 g	13 (5.3)	9 (3.5)	0.323
< 10th centile	34 (13.9)	48 (18.8)	0.146
< 3rd centile	8 (3.3)	13 (5.1)	0.316
High level neonatal care for > 48 h	71 (29.1)	75 (29.3)	0.961

(N (%) unless otherwise stated), *BMI (body mass index), PMR (perinatal mortality ratio)*.

[*] The groups were compared using the Student's *t*-test.

[†] These characteristics were used for adjustment only in exploratory analyses.
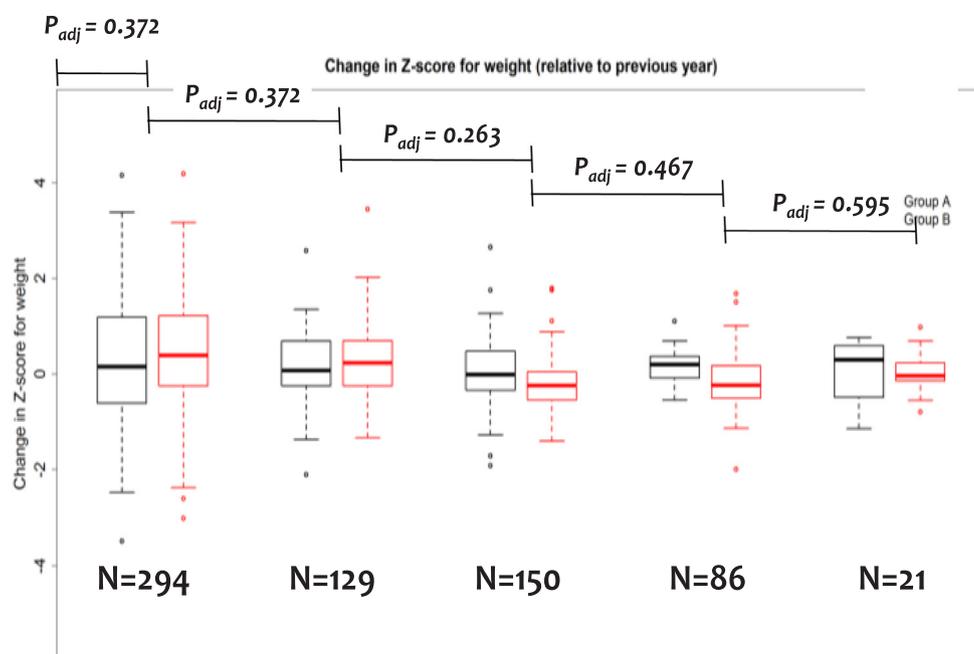
Fig. 2. Change in z-score for weight' relative to birth or the year prior, until age 5 years (as applicable), for children in the 'less tight' (in black) vs. 'tight' (in red) control groups. The N children evaluated at each time point relative to the year before is presented at the bottom of each set of box plots which represent the median, interquartile range, and outliers for each group. The p values for 'less tight' vs. 'tight' control comparisons are from mixed effects linear regression with centre and subject as random effects, and following adjustment for the maternal pre-randomisation factors of type of hypertension, type of antihypertensive, and maternal body mass index (BMI). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

could not be accounted for by maternal adverse outcomes [i.e., no serious maternal complications, and similar rates of severe hypertension (6/15, 40.0% in 'less tight' vs. 7/20, 35.0% in 'tight'; $p = 0.76$) and pre-eclampsia (9/15, 60.0% vs. 7/20, 35.0%, respectively; $p = 0.14$)], maternal mental health [e.g., postnatal antidepressant use (1/15, 6.7% vs. 1/20, 5.0%, respectively; $p = 1.0$)], or livebirth characteristics [i.e., preterm birth (5/15, 33% vs. 2/20, 10.0%, respectively; $p = 0.11$), birthweight < 10th centile (2/15, 13.3% vs. 0/20, respectively, ; $p = 0.18$), or high level neonatal care for > 48 h (5/15, 33% vs. 3/20, 15.0%, respectively; $p = 0.25$)]. The difference also remained when analyses were restricted to the 16 children who also had DNA samples taken (199.8 [33.5, 519.8] vs. 1174.7 [743.8, 1303.2] ng/g, respectively; $p = 0.02$). There was no difference in the distribution of post-randomisation antihypertensive exposure among babies in 'less tight' (vs. 'tight') control: labetalol (4/15, 26.7% vs. 12/20, 60.0% respectively), methyldopa (6/15, 40.0% vs. 5/20, 25.0%), another antihypertensive (2/15, 13.3% vs. 2/20, 10.0%), or no antihypertensive (3/15, 20.0% vs.1/20, 5.0%) ($p = 0.198$).

Buccal swab samples for DNA were collected from 41 children, but only 16 (9 in 'less tight' and 7 in 'tight' control) had samples that passed quality control testing. 90.8% of DNA sites were available for analysis. The strongest associations with DNA methylation were seen with child sex, chip, row on the chip, and child's age (Supplementary Fig. S1a and b). The final model for differential methylation analysis was '*allocated trial group ('less tight' or 'tight' control) + infant sex + infant age + chip + sample position*'. No differences in DNA methylation between groups were identified, as indicated by the data being far below the false discovery rate of < 0.05 (Supplementary Fig. S2).

4. Discussion

4.1. Summary of findings

In this follow-up study of a large international trial of differential BP control in pregnancy, there was no compelling impact of 'less tight' (vs. 'tight') control of hypertension on growth before birth (as documented in CHIPS) [21] or rate of growth after birth, as measured in CHIPS-Child. Although babies whose mothers received 'less tight' (vs. 'tight') control of hypertension had both apparent less rapid postnatal growth (as reflected by a smaller change in z-score for weight from birth) and

less HPA axis activation (as reflected by lower hair cortisol levels) at 12 ± 2 months of age, the difference in postnatal growth rate at this time did not reach statistical significance, and hair samples were provided by only a small subset of children (i.e., 35, 8.4% of all participants). Also, any apparent initial reduction in growth rate associated with 'less tight' (vs 'tight') control did not appear to be ongoing, particularly after the age of two years, and, we could demonstrate no epigenetic modifications (as reflected by DNA methylation) associated with 'less tight' (vs. 'tight') control, even when analyses were restricted to the children who provided both hair and buccal swabs for DNA extraction (among whom hair cortisol was still lower in 'less tight' (vs. 'tight') control). Taken together, these findings suggest that if 'less tight' (vs. 'tight') control of non-severe hypertension in pregnancy does have developmental programming effects, they are not compelling, they do not remain obvious over time, and they do not seem to be mediated by changes in gene expression as measured by DNA methylation.

4.2. How findings fit with literature

There is strong evidence from animal experimentation and epidemiological data that prenatal factors can exert developmental programming effects. In relation to human health, what has been lacking for most exposures is experimental validation. To our knowledge, we report on the first randomised trial of BP control in pregnancy (CHIPS) that has examined developmental programming as a follow-up outcome, as we did in CHIPS-Child.

Small body size at birth is associated with lower muscle mass and higher fat to lean body mass ratio later in life [30,31]. As muscle mass is critical in the storage and oxidation of glucose, individuals with high fat to muscle ratio have an elevated risk of insulin resistance and associated metabolic consequences. Consistent with this is the observation that babies born small are at risk of developing cardiovascular diseases in later life [32].

Whether postnatal catch-up growth is a good or bad thing following small size at birth is controversial. Further complicating matters is whether the timing of that catch-up growth may modify benefit or risk. Some literature suggests that early catch-up growth is harmful; more rapid weight gain in the first year of life (particularly in the first three months) was associated with a greater percentage of body fat and reduced insulin sensitivity in young adults [22]. In contrast, early catch-

up before age two years has been demonstrated to be *beneficial;* among babies of low birthweight, interventions aimed at increasing linear growth in the first two years of life led to significant gains in height and school performance [33], without a negative impact on glucose tolerance [34]. Also, in birth cohort studies, only more rapid growth *after* the age of two years (not before) was associated with elevated cardiovascular risk [33], including coronary heart disease [35] and impaired glucose tolerance [36]. These data highlight the complex and conflicting nature of the literature on the relationship between cardiovascular risk and postnatal growth rate and its timing.

In CHIPS-Child, it may be reassuring that any possible accelerated postnatal growth seen among babies who were exposed to 'tight' control *in utero,* was evident only in the first two years of life. In fact, we cannot rule out a change in the *pattern* of postnatal growth, and if truly different, whether the pattern is better or worse with regards to long-term outcomes. Different patterns of growth have been related to environmental influences such as infant feeding (that did not differ between groups in CHIPS-Child). Primarily formula-fed infants, compared with exclusively breastfed infants, have demonstrated more rapid initial growth followed by slowed growth in the first year of life, with these early differences being much larger (i.e., 0.4–0.6 in z-score) than seen in our study (approximately 0.25 in z-score) [36].

It must be acknowledged that unhealthy lifestyle factors can magnify any elevated cardiovascular risk associated with low birthweight (or reduced growth potential *in utero*). In association with low birthweight, most of the risk of adult type 2 diabetes mellitus was accounted for by unhealthy lifestyle (59%) or an interaction between low birthweight and unhealthy lifestyle (18%), rather than low birthweight itself (22%) [37]. Conversely, following low birthweight, frequent moderate daily exercise (such as undertaking brisk walking three times per week) was associated with a reduced risk of type 2 diabetes [38]. These data are consistent with the known effect of exercise in improving muscular insulin sensitivity.

4.3. Strengths and limitations

Strengths of CHIPS-Child include examination of measures of developmental programming following antenatal measures of BP control; we are not aware of another such study. Also, the CHIPS Trial on which CHIPS follow-up is based, was a high-quality, large, multicentre, international RCT, an additional strength.

An important limitation of our study is the smaller sample size (417 babies) than anticipated (506 babies). Fewer CHIPS centres (59/94, 62.8% centres rather than 75/94, 80% anticipated) chose to participate in CHIPS-Child, and our follow-up rate (73.2%) was suboptimal. Many sites' research ethics boards required CHIPS-Child to be submitted as a new proposal distinct from CHIPS, such that by the time that local approvals were in place, only 92/500 children followed up were still eligible for a CHIPS-Child study visit at 12 ± 2 mos corrected postgestational age. This was the point at which biological samples were collected for hair cortisol and epigenetic analyses, further limiting our ability to examine these endpoints. Second, only about half of women were willing to bring their babies back for a face-to-face visit, because CHIPS-Child was a separate study from CHIPS that did not specify follow-up beyond six weeks postpartum. Third, DNA methylation changes may have been missed because they disappeared over time (which would be reassuring) or they were tissue-specific and for ethical and practical reasons, we had access only to buccal cell DNA. Fourth, although the elevated hair cortisol values in the 'tight' control could not be explained by other confounders, other environmental factors that were not measured may have been causal, and the significant result may represent a type I error as part of many statistical comparisons. Finally, we were able to collect only basic information about infant feeding, with neither calorie counts nor feeding practices after infancy documented, and we lack information about other lifestyle factors, namely activity.

4.4. Conclusions

The results of this study suggest that any developmental programming effects as a result of maternal BP control during pregnancy are not convincing, do not persist over time, and appear to not be mediated by gene expression alterations. For researchers, there are interesting observations in CHIPS-Child related to postnatal growth rate pattern and HPA axis activation that warrant future study, for confirmation and for association with clinical outcomes, particularly paediatric BP and neurodevelopment. For clinicians, CHIPS-Child results do not reveal compelling evidence for developmental programming of either postnatal growth or the HPA axis associated with maternal BP control during pregnancy. Clinicians should look to the clinical findings of CHIPS to guide clinical practice; on balance, the best approach appears to be 'tight' control of non-severe pregnancy hypertension for maternal benefit, without evidence of harm to the baby in the short-term (as shown in CHIPS) or long-term (as demonstrated here in CHIPS-Child). Of course, for all individuals, healthful diet and activity throughout life are key to the avoidance of cardiovascular disease long-term.

Previous presentation

Presented as an abstract at ISSHP 2016: Laura Ann Magee, Anne Synnes, Peter von Dadelszen, Anna Hutfield. CHIPS-Child: Testing the developmental programming hypothesis in the offspring of the CHIPS trial: Randomized trials, Pregnancy Hypertension 2016; 6:143.

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Conflicts of interest

None.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.preghy.2018.04.021.

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