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Long term alterations of growth after antenatal steroids in preterm twin pregnancies

Abstract

Objectives: To compare the long-term effects of antenatal betamethasone (ANS, ≤16 mg, =24 mg and >24 mg) in twins on infant and childhood growth.
Methods: A retrospective cohort follow up study among 198 twins after ANS including three time points: U1 first neonatal examination after birth and in the neonatal period; U7 examination from the 21st to the 24th month of life and U9 examination from the 60th to the 64th month of life using data from copies of the children's examination booklets. Inclusion criteria are twin pregnancies with preterm labor, cervical shortening, preterm premature rupture of membranes, or vaginal bleeding, and exposure to ANS between 23+5 and 33+6 weeks. Outcome measures are dosage-dependent and sex-specific effects of ANS on growth (body weight, body length, head circumference, body mass index and ponderal index) up to 5.3 years.
Results: Overall, 99 live-born twin pairs were included. Negative effects of ANS on fetal growth persisted beyond birth, altered infant and childhood growth, independent of possible confounding factors. Overall weight percentile significantly decreased between infancy and early childhood by 18.8%. Birth weight percentiles significantly changed in a dose dependent and sex specific manner, most obviously in female-female and mixed pairs. The ponderal index significantly decreased up to 42.9%, BMI index increased by up to 33.8%.
Conclusions: ANS results in long-term alterations in infant and childhood growth. Changes between infancy and early childhood in ponderal mass index and BMI, independent of dose or twin pair structure, might indicate an ANS associated increased risk for later life disease.
Synopsis: First-time report on long-term ANS administration growth effects in twin pregnancies, showing persisting alterations beyond birth in infant and childhood growth up to 5.3 years as potential indicator of later life disease risk.

Keywords: antenatal betamethasone; antenatal steroid (ANS); dosage; early childhood; follow up; infancy; sex; twin pregnancy.

Introduction

In pregnancies threatened with preterm birth, antenatal steroid (ANS) treatment to induce fetal lung maturation and to reduce neonatal morbidity and mortality is common practice [1] and appropriate alternatives currently do not exist [2]. We have previously shown that ANS treatment in normally grown singleton and twin pregnancies reduced in a dose-dependent and sex-specific manner fetal weight gain, length and head circumference compared with gestational age-matched controls [3, 4]. ANS doses escalation even increased the negative effects on fetal growth, but did not improve neonatal morbidity and mortality at birth. Although birth weight is only a rough surrogate marker for hormonal or nutritional changes in the intrauterine environment, impaired fetal growth is associated with a number of long-term developmental and health implications [5, 6]. Exposure to high levels of glucocorticoids *in utero* is amongst the proposed mechanisms of perinatal programming [5, 7, 8]. The implications for ANS

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are still not fully understood. There are still ongoing trials and research projects in singleton pregnancies, investigating all different kinds of aspects regarding ANS treatment such as which type of glucocorticoid and which formula should be used, which dosages and time intervals between injections are preferable, which gestational age would benefit most and what kind of long term implications are associated even with reduced ANS dosages [8].

In multiple pregnancies, the different pharmacokinetics of ANS compared to singletons, the increased total fetal mass and increased volume distribution in twins led to the discussion that the current glucocorticoid dose applied might be too low to induce fetal lung maturation [9–13]. The effects of antenatal lung maturation with glucocorticoids in multiple pregnancies still remains unclear as trials in twins are limited and evidence is lacking [1, 9–15]. Especially follow up studies on long term changes are missing.

Therefore, the present study's objective was to investigate in twin pregnancies ANS dosage and sex-specific long-term effects on infant and child growth development up to 5.3 years of age.

Materials and methods

Prospective cohort study of twins born between December 1993 and January 2011 in a tertiary referral center at the Clinic of Obstetrics, Charité University Hospital Berlin, Germany. Details on the original birth cohort has been described previously [4]. Briefly, cases were defined as twin pregnancies with the diagnosis of preterm labor with symptomatic contractions and cervical ripening, premature rupture of membranes or vaginal bleeding and exposed to antenatal betamethasone (Celestan®, MSD GmbH, Haar, Germany) between 23 weeks plus five days of pregnancy (23^{+5} weeks) and 33^{+6} weeks and delivered between 25^{+0} and 36^{+6} weeks. Exclusion criteria were: high numbered multiple pregnancies, twin-to-twin transfusion syndrome, intrauterine death, malformations, chromosomal anomalies or other fetal or maternal diseases and pathological umbilical or uterine doppler findings. Fetuses with sonographically estimated fetal weight below the 10th centile at the time of the initial betamethasone treatment were defined as small for gestational age and excluded from the study to rule out possible growth restricting by an already existing placental insufficiency [4]. Estimated date of delivery was corrected by first trimester ultrasound when the difference between due dates by last period and that by early sonography was more than seven days.

Patients were contacted by telephone and post (Supplemental Material S1) and asked to participate in this follow up study. Follow-up examination included three time points: (U1) first neonatal examination after birth and neonatal period up to 10 days after birth (Supplemental Material S2) using data from the clinic charts, (U7) examination from the 21st to the 24th month of life and (U9) examination from the 60th to the 64th month of life using data from copies of the children's examination booklets [16] sent back by the parents (Supplemental

Material S3). For the documented anthropometric data, percentiles were calculated using the Ped (Z) pediatrician calculator [17]. This web calculator is based on data from Kromeyer-Hauschild et al. for pediatric percentiles of infants, children and adolescents for height, weight and BMI [18]. In addition, percentiles for 0–6 year old children were derived for a variety of somatic parameters according to Hesse et al. [19–21]. For the head circumference percentiles, the data of the 'Zurich Longitudinal Studies' (1955–2009) were used [22].

As reported previously, the protocol for steroid administration changed over time at our institution according to different local and international clinical recommendations in effect during the study period [4]. The betamethasone treatment regimen ranged from 2×8 mg (≤16 mg) or 2×12 mg (=24 mg) given once during pregnancy, to repeated doses of betamethasone (8 mg weekly or 2×12 mg every second week) when the diagnosis of preterm labor still persisted (collectively grouped as ≥24 mg). Betamethasone dosage effects were analyzed among three different dosages: ≤16 mg, 24 mg and >24 mg. Gender specificity was tested for male-male, female-female and mixed-pairs of twins. The Charité Hospital Ethics Review Committee approved the trial (EA2_111_15). All participants gave written informed consent before the study began.

Statistics

Crossed items in the sent copies of the children's examination booklets were interpreted as true, unchecked items as inaccurate and thus transferred to an Excel database. Statistical analyzes were performed using the SPSS 25 software (IBM, SPSS Statistics, version 25, IBM Corporation, Armonk, NY, USA). Data were tested for normality assessing the histogram and using the Shaipiro-Wilks test. To analyze the long-term effects of antenatal betamethasone on anthropometrics (body weight, head circumference, body length, body mass index, triponderal mass index [23] and body mass index), in terms of steady state characteristics, the unifactorial ANOVA with Kruskal-Wallis post hoc test was performed for two or more independent samples. For repeated measures a MANOVA and Friedman post hoc test was used to identify possible significant effects of different betamethasone dosages on growth trajectories (growth percentiles, ponderal index and body mass index) in follow up data between U1 and U9 time points. Data are reported as median and interquartile range or mean and standard deviation, as appropriate. Possible confounders were identified (Supplemental Material S4) and significant results were controlled for major confounders by analysis of covariance (ANCOVA). Statistical analyses were performed for overall, male-male, female-female and mixed-pairs. For all tests, statistical significance was set at p<0.05.

Results

Study population

In total, the response rate was 29.9% and 198 children of twin pregnancies, who had received antenatal betamethasone treatment and were born between 25^{+0}

and 36^{+6} weeks of gestation, were included into the study (Figure 1). Seventy one (71.7%) women remained pregnant seven or more days after the initial betamethasone treatment. Sixty children (30.3%) were born after 34^{+0} weeks of gestation. Maternal characteristics (Supplemental Material S4) were not associated with the observed changes in anthropometrics after betamethasone exposure in multivariate analysis. Neither the number and distribution within the twin-pair structure nor its relation to the dosis-group was significant different (data not shown).

Overall effects

Overall weight percentile significantly decreased between U1 and U7/9 (U1: 45.5±28.1 centile vs. U9: 37.0±28.2 centile p=0.001; U7: 38.9±27.0 centile vs. U9: 37.0±28.2 centile p=0.027, Figure 2A). The length percentile increased between U7 and U9 (U7: 43.3±31.0 vs U9: 48.0±31.0 p=0.001). The ponderal index as a marker for fat levels, continuously decreased between U1, U7 and U9 (U1: 22.6±2.7 vs. U7: 18.3±1.9 vs. U9: 13.1±1.1 p<0.001, Figure 2D). The BMI index significantly increased between infancy and childhood (U1: 9.7±1.5 vs. U7: 15.7 ± 1.4 / U1 vs. U9: 14.6±1.3 p<0.001, Figure 2D).

Dosage effects

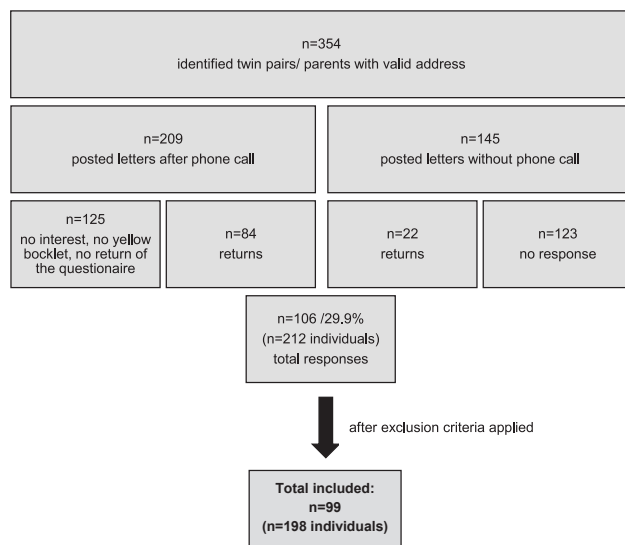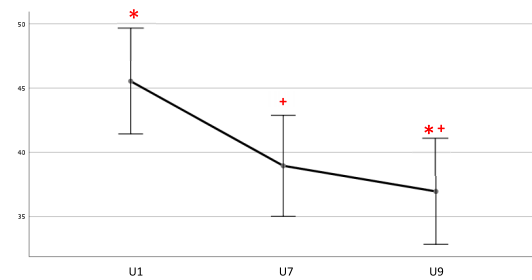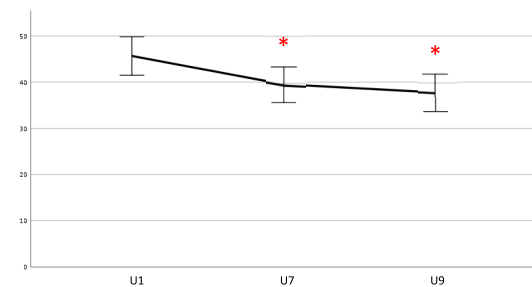Anthropometrics did not significantly differ at U1, U7 or U9 according to the betamethasone dose (Table 1).

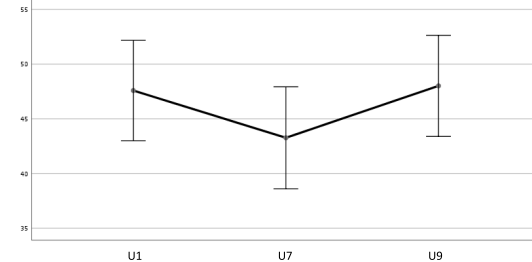**Figure 1:** Patient recruitment and study cohort.
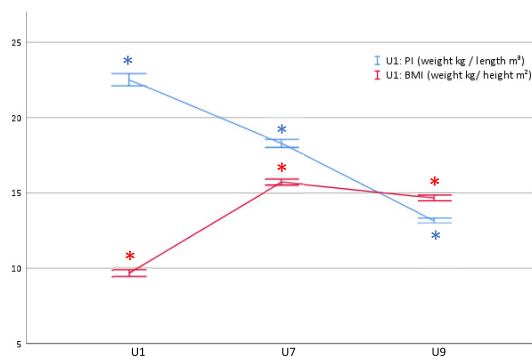

Figure 2: (A–D) Effects of antenatal bethamethasone on growth trajectories.
Data were analyzed by MANOVA for repeated measures followed by Friedman post hoc test. Significant differences between time points are indicated by stars/crosses. Data are presented as mean±95%CI.

Betamethasone dose related changes in growth trajectories between U1 and U9 were observed. In children which had received ≤16 mg betamethasone during pregnancy, weight

Table 1: Betamethasone dosage effects on anthropometrics.

U1	≤16 mg	24 mg	>24 mg	p-Value[a]
Weight, g[+]	1816.0±563.6	1745.7±(610.6)	1746.0±480.9	0.830
	n=96	n=78	n=24	
Weight percentile[++]	**44.2 (52.4)[b]**	42.1 (36.9)	25.1 (52.9)	0.210
	n=96	n=78	n=24	
Length, cm[++]	43.0 (5.0)	43.0 (7.0)	44.0 (4.0)	0.406
	n=87	n=77	n=21	
Length percentile[++]	43.9 (53.7)	**48.9 (57.1)[d]**	51.4 (68.8)	0.919
	n=87	n=77	n=21	
Head circ., cm[++]	31.0 (3.0)	30.0 (5.0)	31.0 (2.0)	0.234
	n=84	n=75	n=18	
Head circ. percentile[++]	54.1 (56.4)	51.1 (51.0)	41.4 (55.4)	0.436
	n=84	n=75	n=18	
Ponderal index[++]	**22.8 (3.0)[f]**	**22.2 (3.9)[g]**	**22.3 (4.7)[h]**	0.138
	n=87	n=77	n=21	
BMI[+]	**9.9±1.4[j]**	**9.4±1.5[k]**	**9.4±1.7[l]**	0.154
	n=87	**n=77**	**n=21**	
U7	≤16 mg	24 mg	>24 mg	p-Value[a]
Weight, g[+]	11848.7±1507	11402.9±1383.5	11761.3±1383.5	0.181
	n=95	n=76	n=23	
Weight percentile[++]	**38.0 (50.0)[c]**	33.0 (43.3)	49.0 (47.0)	0.118
	n=95	n=76	n=23	
Length, cm[++]	86.0 (5.0)	85.0 (5.0)	86.0 (7.0)	0.390
	n=95	n=76	n=23	
Length percentile[++]	39.0 (55.0)	**31.5 (50.8)[e]**	43.0 (70.0)	0.558
	n=95	n=76	n=23	
Head circ., cm[+]	48.3±1.8	48.0±1.9	48.9±1.7	0.172
	n=93	n=76	n=23	
Head circ. percentile[++]	35.0 (53.5)	28.5 (55.8)	64.0 (56.0)	0.067
	n=93	n=76	n=23	
Ponderal index[+]	**18.5±1.6[f]**	**18.2±2.2[g]**	**18.0±1.4[i]**	0.286
	n=95	n=76	n=23	
BMI[+]	**15.9±1.3[j]**	**15.5±1.6[k]**	**15.6±1.0[l]**	0.185
	n=95	**n=76**	**n=23**	
U9	≤16 mg	24 mg	>24 mg	p-Value[a]
Weight, g[++]	18100 (4150)	18000 (3350)	18900 (3800)	0.682
	n=85	n=77	n=23	
Weight percentile[++]	**27.0 (55.0)[b,c]**	33.0 (45.5)	46.0 (57.0)	0.826
	n=85	n=77	n=23	
Length, cm[+]	111.7±5.3	111.1±5.1	112.8±5.3	0.384
	n=85	n=77	n=23	
Length percentile[++]	40.0 (56.5)	**46.0 (49.0)[d,e]**	61.0 (65.0)	0.311
	n=85	n=77	n=23	
Head circ., cm[++]	51.0 (2.0)	51.0 (2.0)	51.7 (3.6)	0.701
	n=14	n=54	n=8	
Head circ. percentile[++]	45.0 (52.3)	33.5 (46.3)	62.5 (91.0)	0.753
	n=14	n=54	n=8	
Ponderal index[+]	**13.3±1.2[f]**	**13.1±1.2[g]**	**12.8±1.0[h,i]**	0.159
	n=85	n=77	n=23	
BMI[++]	**14.8 (2.2)[j]**	**14.5 (1.7)[k]**	**14.4 (1.6)[l]**	0.379
	n=85	**n=77**	**n=23**	

Data presented as mean[+] (standard deviation [SD]) and median[++] (interquartile range [IQR]) for normally and non-normally distributed data, respectively. [a]Kruskal–Wallis test, asymptomatic significance. Growth trajectories – weight percentile: ≤16 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [b]U1 vs. U9 p=0.001, [c]U7 vs. U9 p=0.012; Length percentile: 24 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [d]U1 vs. U9 p=0.037, [e]U7 vs. U9 p<0.001; Head circumference percentile: no sig. differences; Ponderal index: ≤16 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [f]U1 vs. U7 vs. U9 p<0.001; 24 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [g]U1 vs. U7 vs. U9 p<0.001; >24 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [h]U1 vs. U9 p<0.001, [i]U7 vs. U9 p<0.001, BMI ≤16 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [j]U1 vs. U7 vs. U9 p<0.001; 24 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [k]U1 vs. U7 vs. U9 p<0.001; >24 mg: MANOVA for repeated measures p<0.001 (Friedman's two-way analysis of variance by ranks): [l]U1 vs. U7 vs. U9 p<0.005. Bold values indicate significant differences within dosis groups across time points U1 vs. U7 vs. U9.

percentiles significantly decreased between U1 and U9 (Table 1). In children who had received 24 mg betamethasone, length percentiles significantly increased between U7 and U9 (p=0.037), but were still lower compared to U1 (p<0.001). Head circumference percentiles were not affected by the applied betamethasone dose and did not significantly change between U1 and U9 (Table 1). Similar to the observed overall patterns of body mass index and ponderal index (Figure 2D), in all three dosis regimes, ponderal index continuously decreased between U1 and U7 and U9 (p<0.001) and the body mass index significantly increased between U1 and U7 and U9 independent of the dose (Table 1).

Sex effects

Effects of antenatal betamethasone treatment on anthropometrics were sex dependent. Female-female twin pairs were significantly lighter and smaller (lower weight and length percentile at U1) and had a decreased ponderal index (U7) compared with mixed twin pairs, but were not significant different anymore at later time points (Table 2). Head circumference percentiles were significantly smaller in females compared to males at U7, which was consistent within the mixed twin pairs at U7 (Table 2). At U9, no sex dependent differences in anthropometrics could be observed.

Growth trajectories across the three time points significantly changed and weight percentiles in female-female pairs (U7 vs. U9) and mixed pairs (U1 vs. U7 and U1 vs. U9) significantly decreased (Table 2). Between U1 and U9, head circumference percentiles significantly increased in female-female pairs, but was not significantly different from male-male or mixed pairs. The ponderal index continuously decreased between U1 and U7 and U9, independent of twin pair structure. The body mass index significantly increased between U1 and U7 and decreased between U7 and U9, however still significantly higher compared to U1 (Table 2).

Combined effects

Analyzing the combined effect of antenatal betamethasone dose and twin pair structure on anthropometrics, birth weight percentiles significantly decreased only in the ≤16 mg dosis group in female-female pairs between U7 and U9 (Figure 3A). In the >24 mg group, weight percentiles significantly increased between U1 and U7 (Figure 3A). In male-male and mixed pairs, no significant differences in birth weight percentiles were observed. Body length percentiles significantly increased in female-female pairs between U1 and U7 in the ≤16 mg group. In mixed pairs, body length percentiles decreased between U1 and U7 in all three dosage groups, but significantly increased again between U7 and U9 in the 24 mg group (Figure 3B). In the male-male pairs no significant differences in body length percentiles were observed. Head circumference percentiles significantly increased only in female-female pairs received 24 mg betamethasone, no changes were observed in male-male or mixed pairs (Figure 3C).

The ponderal index as a measure of fat levels in childhood and adolescence significantly decreased between U1 and U9, independent of twin pair structure or betamethasone dose (Figure 3). In male-male and female-female pairs the ponderal index decreased up to 43.5%, in mixed pairs up to 41.8%. The BMI index however increased between U1 and U7, independent of twin pair structure and betamethasone dose (Figure 4). Between U7 and U9 body mass indices decreased again in ≤16 and 24 mg bethamethasone dosis groups, but still being higher compared to U1.

Discussion

To our knowledge, this is the first study to investigate long-term effects of different antenatal betamethasone doses in twin pregnancies. The fetal growth restricting effects of antenatal betamethasone treatment in this follow up study of the original twin-preterm birth cohort, persisted beyond

Table 2: Antenatal betamethasone and the effects of anthropometry with respect to twin pair structure.

U1	Male-male	Female-female	Mixed	p-Value[a]	Mixed		p-Value[b]
					Male	Female	
Weight, g[++]	1755.0 (775)	1782.5 (460)	1907.5 (909)	0.158	1932.5±677.1	1830.0±658.8	0.497
	n=66	n=66	n=66		n=33	n=33	
Weight percentile[++]	41.8 (46.6)	29.7 (43.0)[a]	52.0 (42.1)[a,f,g]	**0.009**	59.9 (51.9)[o]	45.1 (30.6)	0.352
	n=66	n=66	n=66		n=33	n=33	
Length, cm[++]	42.0 (6.0)	43.0 (5.0)	44.0 (7.0)	0.126	44.0 (7.0)	44.0 (7.0)	0.778
	n=59	n=64	n=62		n=31	n=31	
Length percentile[++]	36.8 (58.7)	34.6 (56.6)[d]	55.9 (52.3)[b,i,j]	**0.008**	69.5 (38.6)[p]	53.7 (49.3)[r]	0.356
	n=59	n=64	n=62		n=31	n=31	
Head circ., cm[++]	30.0 (4.0)	31.0 (3.0)[k]	31.0 (4.0)	0.312	31.0 (4.0)	31.0 (4.0)	0.469
	n=58	n=62	n=57		n=28	n=29	
Head circ. percentile[++]	50.3 (52.9)	40.5 (53.4)	63.0 (52.2)	0.088	65.7 (57.7)	53.4 (48.2)	0.371
	n=58	n=62	n=57		n=28	n=29	
Ponderal index[+]	22.5±2.7[l]	22.7±2.9[m]	22.5±2.6[n]	0.983	22.7±2.7[t]	22.3±2.5[u]	0.486
	n=59	n=64	n=62		n=31	n=31	
BMI[+]	9.5±1.5[v]	9.6±1.3[w]	9.8±1.7[x]	0.309	10.0±1.7[y]	9.7±1.7[z]	0.251
	n=59	n=64	n=62		n=31	n=31	

U7	Male-male	Female-female	Mixed	p-Value[a]	Mixed		p-Value[b]
					Male	Female	
Weight, g[+]	11811.8±1640.0	1138.6±1343.4	11776.8±1296.4	0.144	11834.4±1484.2	11719.2±1097.4	0.788
	n=66	n=62	n=66		n=33	n=33	
Weight percentile[++]	38.0 (49.3)	37.5 (46.3)[e]	38.0 (47.0)[f]	0.940	34.0 (51.5)	44.0 (37.5)	0.488
	n=66	n=62	n=66		n=33	n=33	
Length, cm[++]	86.2 (6.1)	86.0 (5.0)	85.0 (6.0)	0.440	86.1±4.1	85.5±3.9	0.471
	n=66	n=62	n=66		n=33	n=33	
Length percentile[++]	36.0 (65.3)	52.0 (46.8)[h]	29.5 (50.5)[i]	0.007	20.0 (58.0)[p,q]	32.0 (43.5)[r,s]	0.686
	n=66	n=62	n=66		n=33	n=33	
Head circ., cm[++]	49.0 (2.3)[a]	47.2 (1.7)[a]	48.0 (1.6)	<0.001	49.0 (1.7)	48.0 (1.3)	0.019
	n=66	n=60	n=66		n=33	n=33	
Head circ. percentile[++]	35.5 (56.8)	30.5 (58.0)	33.0 (42.5)	0.821	31.0 (47.5)	35.0 (41.5)	0.500
	n=66	n=60	n=66		n=33	n=33	
Ponderal index[+]	18.5±2.2[l]	17.7±1.4[b,m]	18.6±1.7[b,n]	**0.006**	18.5±1.7[t]	18.8±1.6[u]	0.270
	n=66	n=62	n=66		n=33	n=33	
BMI[+]	15.9±1.6[c,v]	15.3±1.2[c,d,w]	16.0±1.2[d,x]	**0.012**	15.9±1.3[y]	16.0±1.1[z]	0.505
	n=66	n=62	n=66		n=33	n=33	

U9	Male-male	Female-female	Mixed	p-Value[a]	Mixed		p-Value[b]
					Male	Female	
Weight, g[++]	18000.0 (4450.0)	17900.0 (3775.0)	18400.0 (3350.0)	0.446	18550.0 (4225.0)	18400.0 (2875.0)	0.859
	n=57	n=64	n=64		n=32	n=32	
Weight percentile[++]	27.0 (59.5)	31.0 (46.8)[e]	35.0 (42.5)[g]	0.270	42.0 (46.8)[o]	33.0 (47.0)	0.615
	n=57	n=64	n=64		n=32	n=32	
Length, cm[++]	111.1 (6.8)	112.0 (7.0)	112.0 (9.1)	0.995	111.5±5.9	111.6±5.7	0.982
	n=57	n=64	n=64		n=32	n=32	
Length percentile[++]	36.0 (57.0)	46.0 (51.3)[h]	51.5 (31.8)[j]	0.560	45.5 (69.0)[q]	57.0 (52.8)[s]	0.865
	n=57	n=64	n=64		n=32	n=32	
Head circ., cm[++]	52.0 (2.5)	51.0 (1.8)	50.5 (1.0)	0.126	50.6±2.1	50.3±1.2	0.375
	n=25	n=24	n=27		n=14	n=13	
Head circ. percentile[++]	61.0 (73.0)	58.5 (52.8)[k]	31.0 (38.0)	0.443	38.4±29.7	38.6±26.2	0.685
	n=25	n=24	n=27		n=14	n=13	
Ponderal index[++]	13.4 (1.7)[l]	12.8 (1.1)[m]	13.2 (1.9)[n]	0.115	13.2±1.2[t]	13.6±1.5[u]	0.506
	n=57	n=64	n=64		n=32	n=32	
BMI[++]	14.8 (2.3)[v]	14.4 (1.4)[w]	14.9 (1.4)[x]	0.161	14.7±1.3[y]	15.1±1.5[z]	0.308
	n=57	n=64	n=64		n=32	n=32	

[a]Kruskal-Wallis test, [c]pairwise comparison p=0.007, [d]pairwise comparison p=0.006. Data presented as mean[+] (standard deviation [SD]) and median[++] (interquartile range [IQR]) for normally and non-normally distributed data, respectively. [a]Kruskal–Wallis test, asymptomatic significance. [b]Mann Whitney U-test; Growth trajectories – weight percentile: female-female: MANOVA for repeated measures p=0.024 (friedman's two-way analysis of variance by ranks): [e]U7 vs. U9 p=0.023; mixed MANOVA for repeated measures p=0.005 (friedman's two-way analysis of variance by ranks): [f]U1 vs. U7 p=0.031, [g]U1 vs. U9 p=0.008; length percentile: female-female: MANOVA for repeated measures p=0.040 (friedman's two-way analysis of variance by ranks): [h]U7 vs. U9 p=0.039; mixed MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [i]U1 vs. U7 p<0.001, [j]U1 vs. U9 p<0.001; head circumference percentile: female-female: MANOVA for repeated measures p=0.021 (friedman's two-way analysis of variance by ranks): [k]U1 vs. U9 p=0.017; ponderal index: male-male: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [l]U1 vs. U7 vs. U9 p<0.001; female-female: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [m]U1 vs. U7 vs. U9 p<0.001; mixed: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [n]U1 vs. U7 vs. U9 p<0.001; BMI: male-male: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [v]U1 vs. U7 vs. U9 p<0.05; female-female: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [x]U1 vs. U7 vs. U9 p<0.005, mixed: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [y]U1 vs. U7 vs. U9 p<0.005, Growth trajectories within mixed pairs – weight percentile: males: MANOVA for repeated measures p=0.023 (friedman's two-way analysis of variance by ranks): [o]U1 vs. U9 p=0.018; length percentile: males: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [p]U1 vs. U7 p<0.001, [q]U7 vs. U9 p=0.001; females: MANOVA for repeated measures p=0.004 (friedman's two-way analysis of variance by ranks): [r]U1 vs. U7 p=0.035, [s]U7 vs. U9 p=0.006; ponderal index: males: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [t]U1 vs. U7 vs. U9 p<0.05; females: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [u]U1 vs. U7 vs. U9 p<0.005; BMI: males: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [y]U1 vs. U7 vs. U9 p<0.01; females: MANOVA for repeated measures p<0.001 (friedman's two-way analysis of variance by ranks): [z]U1 vs. U7 vs. U9 p<0.005. [a]Kruskal-Wallis test, [b]pairwise comparison p<0.001,[c]pairwise comparison p=0.005, [d]pairwise comparison p=0.047, [e]pairwise comparison p=0.018. Bold values indicate significant differences within dosis groups across time points U1 vs. U7 vs. U9.

birth and resulted in an alteration in infant and early childhood growth up to 5.3 years of age, independent of possible confounding factors. Birth weight percentiles significantly changed in a dose dependent and sex specific manner, most obviously in female-female and mixed pairs. Betamethasone associated changes between infancy and early childhood in ponderal mass index and BMI indicate an antenatal betamethasone associated increased risk for later life disease.

Previously, we have shown in singletons that betamethasone administration was associated with a dose-dependent reduction in birth weight, even after adjusting for major confounders, sex and gestational age at treatment [3]. A single course of betamethasone in pregnant women was related to a symmetrical growth reduction with significantly decreased birth weight (–18.2%), head circumference (–8.6%) and body length (–6.0%) [24]. Although birth weight is only a rough surrogate marker for hormonal or nutritional changes in the intrauterine environment, impaired fetal growth is associated with a number of long-term developmental and health implications [5, 6]. Also in twin pregnancy, antenatal glucocorticoid treatment reduced fetal weight gain, length and head circumference in a dose-dependent and sex-specific manner compared with gestational age-matched controls [4]. Betamethasone dose escalation increased the negative effects on birth weight, head circumference and length. Follow-up estimated fetal weight of normally grown fetuses revealed, that reduced weight gain was directly associated to betamethasone exposure and remained present in pregnancy in the pooled analysis of all twins up to six weeks after betamethasone exposure. Neonatal Apgar scores, umbilical cord blood gases and rates of breathing disturbances were not substantially improved after higher doses of betamethasone treatment and did not improve neonatal morbidity and mortality compared to gestational-age matched controls [4].

The reason for that is unknown. Different pharmacokinetics of betamethasone in multiple pregnancies when compared to singletons have been reported [13]. The half-life of betamethasone in mothers with twin pregnancies was significantly shorter than that in mothers with singleton pregnancies [13]. In addition, increased total fetal mass and increased volume distribution in twins led to the discussion that the current glucocorticoid dose applied might be to low [9–12] and some guidelines even recommend repeat dosing of antenatal corticosteroids [25].

Now, for the first time we could demonstrate, that the negative effects of antenatal glucocorticoid treatment on fetal growth patterns persisted beyond. In this follow up study of the original twin-preterm birth cohort, antenatal glucocorticoid treatment significantly altered postnatal growth trajectories. Overall, weight percentiles significantly decreased between infancy and early childhood by up to 18.8% (U1 vs. U9 p=0.002). By analyzing the effects of different betamethasone doses, the decrease in birth weight and length percentiles between infancy and early childhood was most obvious in the ≤16 and 24 mg
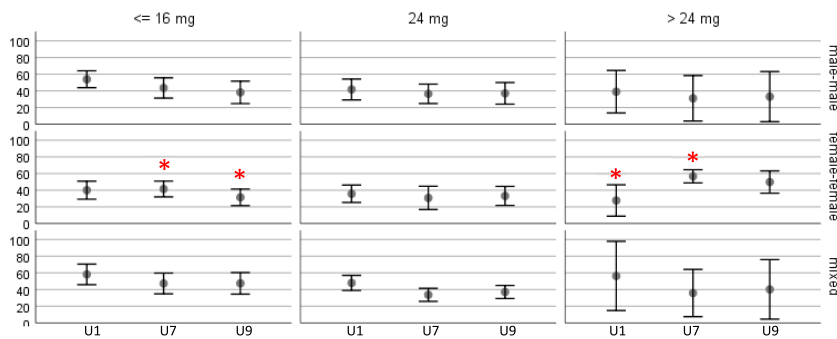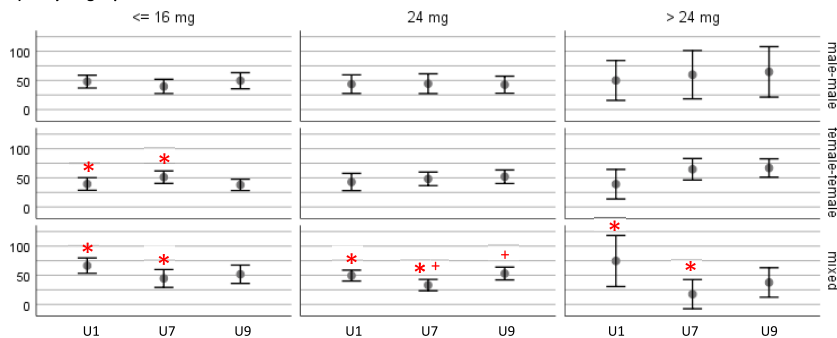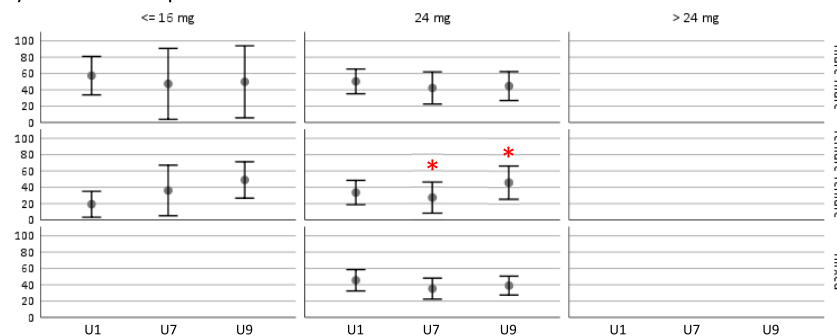
Figure 3: (A–C) Effects of different antenatal bethamethasone dosages on growth trajectories with respect to twin-pair structure.
Data were analyzed by MANOVA for repeated measures followed by Friedman post hoc test. Significant differences between time points are indicated by stars/crosses. Head circumference percentiles in >24. mg and mixed ≤16 mg are not shown due too low numbers. Data are presented as mean±95%CI.

betamethasone dose group and not seen in the ≥24 mg betamethasone dose group. Taking the twin pair structure into account, birth weight percentiles did not change in male-male pairs. In line with our previous observations in singletons [3, 26] and twins [4], sex-specific strategies for adapting to changes in the uterine environment have been described [27] and the present study supports our antenatal findings, that female-female and mixed pairs experience more growth restriction in response to prenatal betamethasone than male-male pairs, which persisted beyond birth and early childhood. Only in female-female pairs which had received ≤16 mg bethamethasone, birth weight percentiles significantly decreased. ≥24 mg betamethasone actually increased weight percentiles in female-female

pairs between U1 and U7 and let us speculate, that with regard to postnatal weight development, the dose of 24 mg antenatal betamethasone might be favorable to prevent growth alterations in preterm twin pregnancies.

Body length percentiles significantly increased overall between infancy and early childhood by up to 10.9% (U7 vs. U9 p=0.037). Body length percentiles in male-male pairs were unaffected by antenatal glucocorticoid treatment. However, in mixed pairs (U1 vs. U7, all three doses) antenatal glucocorticoid treatment resulted in a significant decrease in body length percentiles between U1 and U7, independent of the betamethasone dose.

The ponderal index is a relative stable measure of adipose tissue (fat) levels in childhood and adolescence

A) Ponderal index

B) BMI
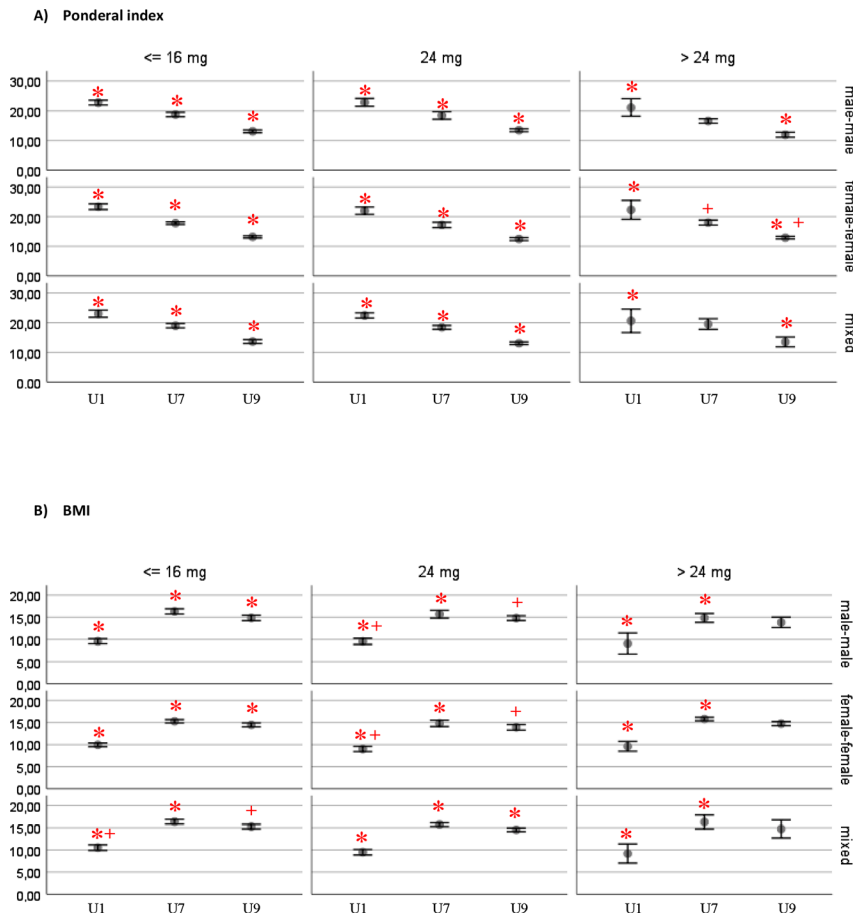
Figure 4: (A–B) Ponderal index (body weight in kg/height in m³) and BMI (body weight in kg/height in m²) in relation to betamethasone dosis group and twin pair structure.
Data were analyzed by MANOVA for repeated measures followed by Friedman post hoc test. Significant differences between time points are indicated by stars/crosses, data are mean±95%CI.

[28]. Rapid gain in ponderal index in infancy has been associated with increased obesity and adverse later outcome such as cardiovascular disease [29, 30]. However, others reported less evidence for the changes in ponderal index in infancy but demonstrated a strong association of rapid BMI change between 8.5 and 10 years of age and associate risk for cardiovascular disease [31]. We therefore analyzed the ponderal index as well as the changes of BMI after antenatal betamethasone in infancy and early childhood up to the age of 5.3 years. Interestingly, the ponderal index significantly decreased between U1 and U9, independent of twin pair structure or betamethasone dose. The BMI index, however, increased between U1 and U9 (≤16 and 24 mg) and between U1 and U7 (≥24 mg), independent of twin pair structure, probably indicative of catch up growth in length. Between U7 and U9, BMI trajectories significantly decreased again in male-male and female pairs with ≤16 mg and mixed pairs with 24 mg. These opposite BMI and ponderal index trajectories between birth and the age of five in twins after antenatal betamethasone treatment were similarly observed earlier in singletons [32] and seem to follow within a 2x standard deviation the

"physiological profile of normalization" of this index. Changes in BMI between 2 and 5 years have been reported to be strongly associated with later adiposity and Howe et al. suggested that BMI changes were also strongly associated with increased fat mass, as well as with a range of cardiovascular risk factors at age 15 [31].

We do acknowledge, that the BMI is associated with both lean and fat mass in young children, however, in infancy and childhood the BMI is an imperfect measure of adiposity. We therefore included the data for the ponderal index and evidence from other cohorts suggests that BMI has similar magnitudes of association to cardiovascular risk factors in childhood as does total fat mass assessed by DXA or waist circumference, suggesting that BMI may adequately assess adiposity in childhood [31].

There are limitations to our study. Uncontrolled data collection and selection bias may be present in the analyses. However, due our large sample size, these effects are unlikely to account for the differences observed. Although we used regression analysis to control for possible confounding variables, the multiple factors affecting growth make comparison of groups difficult and we acknowledge

that additional factors affecting growth trajectories exist. Unique strengths of our study are the large sample size; the exclusion of fetuses with EFW <10th centile at the time of betamethasone treatment; our ability to differentiate between same-sex and mixed-sexed pairs; a broad gestational age range, including both very early and early preterm birth infants and the analysis of different betamethasone dosage regimens.

A web-based calculator was used to calculate the growth percentiles in our study. The reference data behind includes growth patterns only from singletons. However, it has been reported that differences in length/height, weight and BMI between twins and singletons decline during the first 2.5 years, but do not disappear completely. Part of these differences remains even after correcting for premature birth [33]. This has to be taken into consideration when referring to our growth percentiles. The only data, to our knowledge, on growth references for height, weight and body mass index for twins aged 0–2.5 years, have been published in 2008, but using data from twins born between 1986 and 1992, so almost 30 years ago [33]. Having in mind the world pandemic of maternal (and childhood) obesity, well known differences in ethnicity etc., we decided not to use them as control references. We rather used a study design which did not include growth data from respective reference controls (singleton or twin), then analyzing the effects on antenatal betamethasone on postnatal growth development in twins with respect to the dosage within the antenatal betamethasone group.

Conclusions

Antenatal betamethasone treatment in preterm twin pregnancies, to induce lung maturation and improve neonatal morbidity and mortality, resulted in impaired fetal growth and decreased birth weights. The negative effects on fetal growth persisted beyond birth and in infancy. Betamethasone treatment decreased growth trajectories and was associated with a decrease in ponderal index and increase in BMI index at 5.3 years of age. Most sensitive to antenatal betamethasone treatment were female-female and mixed pairs, 24 mg betamethasone appears to avoid respective growth alterations. This, however, should be proven in future investigations. Dose escalation, repetitive and high doses of betamethasone >24 mg betamethasone is not recommended. Further studies are needed to understand the mechanism of antenatal glucocorticoid treatment and its impact on long-term development and morbidity, considering the special needs in twin pregnancies.

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