Neonatal Glucocorticosteroid Treatment Causes Systolic Dysfunction and Compensatory Dilatation in Early Life: Studies in 4-Week-Old Prepubertal Rats

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ABSTRACT

Glucocorticosteroid treatment is widely used to prevent chronic lung disease in premature infants. Recent studies in adult rats, treated with dexamethasone in the neonatal period, report negative long-term effects on the heart and severely reduced life expectancy. We treated neonatal rats with dexamethasone and studied cardiac function after 4 wk (prepubertal age) to investigate whether the late effects as previously described are preceded by detectable alterations in cardiac function at a younger age. Male rat pups ($n = 12$) were injected intraperitoneally with dexamethasone on d 1, 2, and 3 (0.5, 0.3, and 0.1 μg/g) of life. Control pups ($n = 10$) received saline. At 4 wk the animals were anesthetized, and a pressure-conductance catheter was introduced into the left ventricle to measure pressure-volume loops. Cardiac function was measured and pressure-volume relations were determined to quantify intrinsic systolic and diastolic function. Subsequently, hearts were excised for histologic examination. Compared with saline-treated animals, dexamethasone-treated rats had a reduced ventricular weight (270 ± 40 *versus* 371 ± 23 mg, $p < 0.001$) and reduced systolic function (end-systolic elastance: 1.24 ± 0.43 *versus* 2.50 ± 1.39 mm Hg/μL, $p = 0.028$). Cardiac output was maintained and end-diastolic volume was increased (84 ± 23 *versus* 59 ± 19 μL, $p = 0.012$) indicating a state of compensatory dilatation. Heart rate, diastolic function, and systemic vascular resistance were unchanged. Neonatal dexamethasone treatment causes cardiac alterations that can be detected in the prepubertal period and that may precede severe cardiac dysfunction later in life. If our findings are confirmed in humans, this may have consequences for a large patient population and cardiac screening at young age may be indicated to enable secondary prevention. (*Pediatr Res* **58: 46–52, 2005**)

Abbreviations
DEX, dexamethasone
EDV, end-diastolic volume
ESP, end-systolic pressure
ESPVR, end-systolic pressure-volume relationship
LV, left ventricle
PRSW, preload recruitable stroke work
SAL, saline
SW, stroke work
WS, wall stress

Glucocorticosteroids, in particular DEX, are widely used to treat or prevent chronic lung disease in preterm infants (1). Besides their antiinflammatory action, glucocorticosteroids stimulate lung maturation and enhance surfactant production (2,3). Thus, glucocorticosteroid treatment improves pulmonary function and enables early weaning from the ventilator and, consequently, increased survival of extremely premature neonates (4).

In the past two decades DEX therapy in preterm infants has gained wide acceptance. However, despite the short-term beneficial effects, concerns have been raised about long-lasting negative side effects of glucocorticosteroids (5–7). Several studies describe neurodevelopmental impairments in infants treated with glucocorticosteroids in the neonatal period (8–10).

With regard to the cardiovascular system, hypertension and reversible myocardial hypertrophy have been reported (11–13), but possible long-term effects became apparent only recently. De Vries *et al.* (14) showed that rats treated with DEX in the neonatal period had decreased heart weights, as well as hypertrophy and signs of early degeneration of cardiomyocytes in

adulthood. Moreover, preliminary findings indicate a significantly reduced life expectancy (15).

These concerns and reports prompted us to investigate whether the late cardiac side effects are preceded by detectable alterations in cardiac function at a younger age. Early detection would be of great importance for possible early intervention in the large number of individuals previously treated with DEX for prevention of chronic lung disease.

METHODS

Animals. The study protocol was approved by the Animal Research Committee of the University of Leiden. The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No.85–23, revised 1996).

Pregnant Wistar rats (270–300 g) were housed individually and kept under conventional housing conditions. Pups were born on d 21–22 of gestation. On the day of birth (d 0), male pups were selected and randomly divided between treatment and control groups. Treatment and control animals were kept separately and placed with foster mothers in groups of four to six pups. Rat pups in the treatment group were injected intraperitoneally (IP) with DEX using a 3-d tapering dose mimicking the 21-d tapering course used in human preterm infants (14). Consequently, the treated animals received 0.5, 0.3, and 0.1 μg/g body weight DEX on, respectively, d 1, 2, and 3 of life. The animals in the control group received equal volumes (10 μL/g) sterile pyrogen-free saline (SAL). Temperature and humidity were kept constant and the rats had free access to food and water. An artificial 12 h-light/12 h-dark cycle was used. Body weight was measured daily during the first week and weekly from d 7 onward. The rats were weaned on d 21 and hemodynamic studies were performed on d 28 (4 wk) as described below.

Animal preparation. Twenty-two male rats (12 DEX and 10 SAL) were studied. The animals were sedated by inhalation of a mixture of halothane (4%) and oxygen. Subsequently, general anesthesia was initiated by intraperitoneal injection of a fentanyl-fluanisone-midazolam mixture (FFM). The FFM mixture consisted of two parts Hypnorm (Jaussen Pharmaceuticals) (0.315 mg/mL fentanyl + 10 mg/mL fluanisone), one part Dormicum (Roche) (5 mg/mL midazolam), and one part water. This mixture was administered in a dose of 0.4 mL/100 g body weight. Supplemental IP injections (one-third of initial dose) were provided if necessary. The animals were placed on a regulated warming pad to keep body temperature constant, intubated using a 20-G cannula and ventilated with air/oxygen mixture (Fio$_2$ = 0.5, 120 strokes/min) using a pressure-controlled ventilator.

Instrumentation. The animals were placed under a microscope (Carl Zeiss GmbH, Jena, Germany) and the left jugular vein and the right carotid artery were exposed *via* a midline cervical incision. The jugular vein was cannulated for infusion of hypertonic saline to determine parallel conductance (see below). *Via* the carotid artery, a combined pressure-conductance catheter (Millar Instruments, Houston, TX) was introduced and positioned into the LV guided by on-line pressure and volume signals. The abdomen was opened *via* a small incision just below the diaphragm to enable temporary preload reductions by directly compressing the inferior vena cava using a cotton-tipped stick. The pressure-conductance catheter was connected to a Sigma-SA signal processor (CD Leycom, Zoetermeer, The Netherlands) for on-line display and registration of LV pressure and volume signals. All data were acquired using Conduct-NT software (CD Leycom, Zoetermeer, The Netherlands) at a sample rate of 2000 Hz and analyzed off-line with custom-made software.

Conductance catheter method. The conductance catheter method to measure instantaneous LV volume has been developed by Baan *et al.* (16). Recently, miniaturized 1.4-F pressure-conductance catheters have been developed that enable pressure-volume studies in closed-chest small animals (17). The catheter used in this study contains four platinum electrodes, each 0.25 mm in width with interelectrode distances between electrodes 1-2, 2-3, and 3-4, respectively, 0.5 mm, 4.5 mm, and 0.5 mm. A high-fidelity pressure sensor is incorporated between electrodes 2 and 3. A 30 μA, 10 kHz current is applied between electrodes 1 and 4 to generate an intracavitary electric field and the voltage gradient between electrodes 2 and 3 is measured to determine the instantaneous electrical conductance of the blood in the LV. The volume calibration of the conductance measurements was performed *in vitro* as described by Yang *et al.* (18) and parallel conductance was determined by the hypertonic saline method (16,19,20).

Steady state hemodynamic measurements. . After instrumentation, LV pressure-volume signals were acquired in steady state to quantify general hemodynamic conditions: Heart rate (HR), stroke volume (SV), cardiac output

(CO), end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), end-diastolic pressure (EDP), and end-systolic pressure (ESP) were determined. Stroke work (SW) was determined as the area of the pressure-volume loop, and the maximal and minimal rate of LV pressure change, dP/dt$_{MAX}$ and dP/dt$_{MIN}$, and the isovolumic relaxation time constant Tau were calculated. Mean aortic pressure (MAP) was determined from the pressure signal recorded just before the catheter insertion into the LV. Systemic vascular resistance (SVR) was calculated as MAP/CO, without correction for central venous pressure. In addition, we determined effective arterial elastance (E$_A$) as ESP/SV.

Pressure-volume relationships. To obtain indices of systolic and diastolic LV function, we determined pressure-volume relations by recording pressure-volume loops during a gradual preload reduction by gently compressing the inferior caval vein. By this procedure we reduced systolic pressure typically by 20–30 mm Hg within 2 s ($\sim$15 beats). To quantify systolic function, we used the end-systolic pressure-volume relation (ESPVR), the relation between dP/dt$_{MAX}$ and EDV, and the preload recruitable stroke work relation (PRSWR: SW *versus* EDV). The slopes of these linear relations, E$_{ES}$ (end-systolic elastance), S-dP, and S-PR, respectively, are sensitive measures of intrinsic systolic LV function (21–23). In addition, the position of the ESPVR was quantified by its intercept at ESP = 75 mm Hg (ESV$_{75}$), whereas the positions of the dP/dt$_{MAX}$-EDV and SW-EDV relations were quantified by calculating their intercepts at EDV = 70 μL, respectively, dP/dt$_{MAX,70}$ and SW$_{70}$ (24). The values (75 mm Hg and 70 μL) were selected retrospectively as the typical mean values of ESP and EDV for these rats.

For diastolic function, the chamber stiffness constant k was determined by fitting the end-diastolic pressure-volume points with an exponential relation.

Wall stress. Time-varying wall stress, WS(t), was calculated from the LV pressure and volume signals (P(t), V(t)) as described by Arts *et al.* (25): WS(t) = P(t) · (1 + 3 · V(t)/V$_{WALL}$). Ventricular wall volume (V$_{WALL}$) was determined postmortem as described below. Peak WS is the maximum of the WS(t) curve.

Parallel conductance. To determine parallel conductance, we performed intravenous hypertonic saline injections (10% saline, 20 μL) (18,20). Parallel conductance was calculated as the mean of three consecutive assessments.

Echocardiographic measurements. To support the conductance catheter-derived findings, we performed echocardiographic measurements in a small group of additional animals (three DEX- and three SAL-treated rats). The animals were sedated with 1.5% isoflurane and placed in a supine position. Imaging was performed in the parasternal long- and short-axis plane through the shaved anterior chest using a Vingmed echocardiography system FIVE with a 10-MHz phased-array transducer. After two-dimensional imaging, a single M-mode line was directed across the ventricle at the level of the papillary muscles perpendicular to the anterior and posterior walls to determine LV end-systolic and end-diastolic diameters. The measurements were performed by an experienced echocardiographer (R.F.M.B.), who was blinded to the treatment.

Histopathological analysis. After the hemodynamic measurements, the heart was arrested by slowly infusing 1 mL 0.1 M CdCl$_2$ *via* the jugular vein. The chest was opened and the heart excised and immersion fixed in phosphate-buffered formalin 4%. After removal of extracardiac structures and atria, the hearts were weighed and then embedded in paraffin. Subsequently, the hearts were sectioned parallel to the equator in 3-mm slices and stained with hematoxylin and eosin for conventional histopathological analysis. A slice at the level of the papillary muscles was selected for measurement of LV free wall thickness.

Statistics. All data are expressed as the mean ± SD. Comparisons between group means were performed by unpaired *t* tests and statistical significance was defined as $p \leq 0.05$.

RESULTS

Rats. Experiments were performed in dexamethasone-treated (DEX, $n = 12$) and saline-treated male rats (SAL, $n = 10$). Figure 1 shows body weight curves for both groups. No significant differences were found on d 0 and d 1, but from d 2 onward the DEX animals had a significantly lower body weight. At 4 wk the mean body weight for DEX was 75 ± 6 *versus* 85 ± 10 g for SAL ($p = 0.013$).

Hemodynamics. Typical LV pressure and volume signals acquired during steady state and followed by a preload reduction induced by caval occlusion are shown in Figure 2. Mean hemodynamic indices derived from the steady state signals are
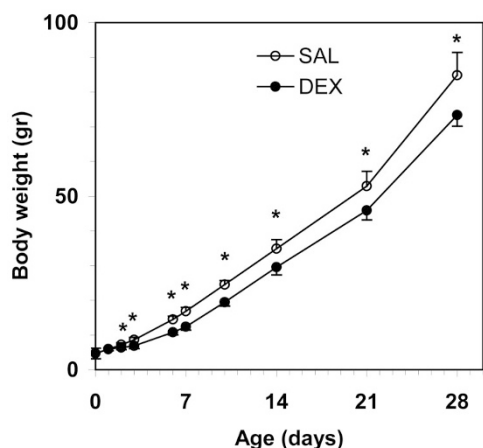
Figure 1. Body weight in SAL- ($n = 10$) and DEX-treated ($n = 12$) rats during 4 wk. Differences were significant ($p < 0.05$) from d 2 onward.
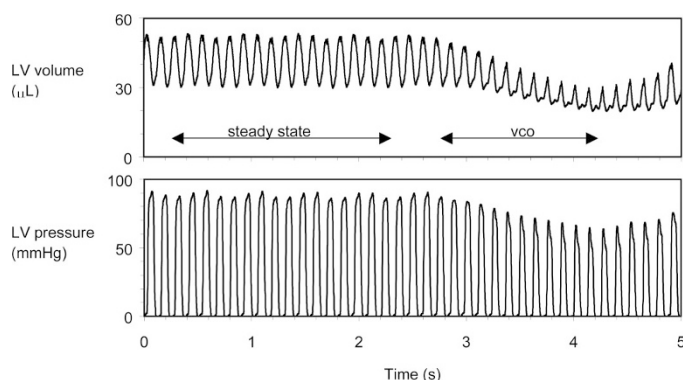

Figure 2. Typical LV pressure and volume signals recorded during steady state, followed by vena caval occlusion (*vco*). Steady state signals were used to determine the various hemodynamic indices as summarized in Table 1. Data acquired during the preload reduction are used to derive the pressure-volume relations as shown in Figure 3 and summarized in Table 2.

given in Table 1. Typical pressure-volume loops obtained during preload reductions for SAL and DEX are depicted in Figure 3, which also shows the derived pressure-volume relationships ESPVR, EDPVR, dP/dt$_{MAX}$-EDV, and PRSWR. The

summarized results for these relations are given in Table 2. The most apparent differences between the groups were found for the ventricular volumes. Compared with the SAL animals, both ESV and EDV were significantly higher (58% and 43%, respectively) in the DEX animals indicating substantial dilatation. Pressure-derived indices (ESP, EDP, Tau, dP/dt$_{MAX}$, and dP/dt$_{MIN}$) were largely unchanged. Heart rate, cardiac output, and ejection fraction were also not significantly altered, although the latter tended to be lower in the DEX animals. SVR and E_A did not change significantly. Calculated peak wall stress was significantly higher. The contractility indices E_{ES} and S-dP (the slopes of the ESPVR and dP/dt$_{MAX}$-EDV relation, respectively) clearly show a reduced intrinsic systolic function in the DEX animals (–50% and –42%, respectively, *versus* SAL). The decrease in S-PR (the slope of the PRSWR) did not reach statistical significance. Reduced contractile state is further supported by the significant rightward and downward shift of the pressure-volume relations as quantified by their intercepts: x-intercept ESV$_{75}$ increased by as much as 56%, whereas y-intercepts dP/dt$_{MAX,70}$ and SW$_{70}$ decreased by 32% and 28%, respectively. The increased chamber stiffness constant k may suggest a slightly reduced diastolic compliance, but the increase did not reach statistical significance. The significant rightward shift of EDPVR (Fig. 3) indicates substantial remodeling in the DEX-treated animals.

Echocardiography. Compared with the SAL rats, the DEX rats showed increased end-systolic (3.0 ± 0.7 *versus* 2.6 ± 0.5 mm) and end-diastolic diameters (4.6 ± 0.5 *versus* 3.9 ± 0.4 mm). The 17% increase in end-diastolic diameters in the DEX rats appears to be fully consistent with the 42% increase in end-diastolic volume found in the invasive studies. The two-dimensional ultrasound images did not allow for reliable and reproducible measurements of long-axis ventricular lengths in all of these rats, but assuming a fixed LV end-diastolic long axis of 7 mm would predict (spheroidal model) an end-diastolic volume of 77 μL in the DEX and 56 μL in the SAL animals.

Histopathology. Heart weight (HW) was significantly lower in the DEX animals compared with the SAL animals (270 ± 40

Table 1. *Hemodynamic indices: SAL- vs DEX-treated rats*

	SAL	DEX	*p* Values
HR (bpm)	509 ± 36	488 ± 46	NS
MAP (mm Hg)	63 ± 11	60 ± 11	NS
ESP (mm Hg)	76 ± 10	74 ± 12	NS
EDP (mm Hg)	3.8 ± 2.7	4.1 ± 2.1	NS
dP/dt$_{MAX}$ (mm Hg/s)	9599 ± 1433	9702 ± 2793	NS
dP/dt$_{MIN}$ (mm Hg/s)	7067 ± 1662	6959 ± 1903	NS
Tau (ms)	9.1 ± 2.0	9.4 ± 1.6	NS
ESV (μL)	34 ± 11	54 ± 18	0.005
EDV (μL)	59 ± 19	84 ± 23	0.012
CO (mL/min)	12.9 ± 4.6	15.0 ± 5.1	NS
EF (%)	43 ± 7	37 ± 8	0.078 (NS)
SW (μL·mm Hg)	2128 ± 982	2481 ± 1106	NS
PWS (mm Hg)	124 ± 21	154 ± 32	0.015
SVR (mm Hg/mL/min)	5.1 ± 1.5	4.3 ± 1.4	NS
E_A (mm Hg/μL)	3.3 ± 1.1	2.6 ± 0.7	0.091 (NS)

Values are mean $\pm$ SD. HR, heart rate; MAP, mean arterial pressure; ESP, end systolic pressure; EDP, end-diastolic pressure; dP/dt$_{MAX}$ and dP/dt$_{MIN}$, maximal and minimal rate of LV pressure change; Tau, time constant of isovolume relaxation; ESV, end-systolic volume; EDV, end-diastolic volume; CO, cardiac output; EF, ejection fraction; SW, stroke work; PWS, peak wall stress; SVR, systemic vascular resistance; E_A, effective arterial elastance.
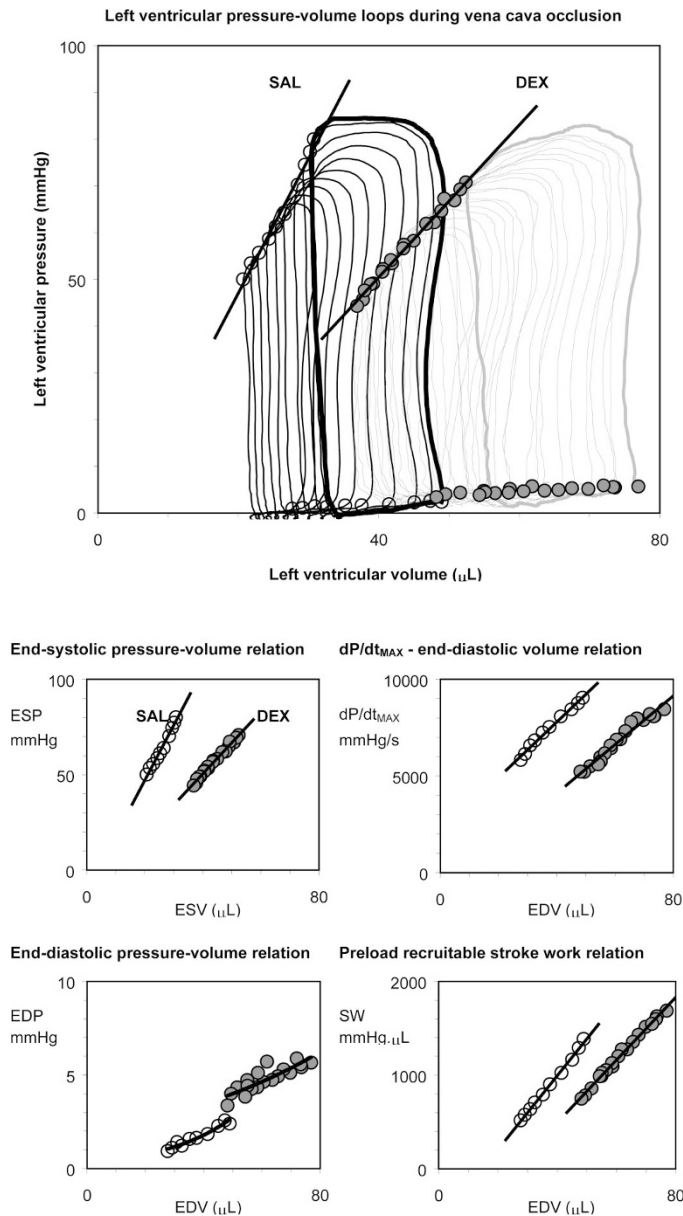
Left ventricular pressure-volume loops during vena cava occlusion

Figure 3. Representative data from a SAL-treated rat (*open circles*) and a DEX-treated rat (*shaded circles*). The *top panel* shows pressure-volume loops during preload reduction. The end-systolic and end-diastolic points are marked and the ESPVR is indicated. The first loop (in bold) represents the preceding steady state condition. The rightward position of the DEX loops on the volume axis indicates substantially enlarged volumes. The reduced slope and rightward shift of the ESPVR indicate a decreased systolic function in DEX compared with SAL. The *lower panels* show the various pressure-volume relations. The ESPVR, the dP/dt_{MAX}-EDV relation, and the PRSWR all show a rightward shift and a decreased slope consistent with reduced contractile state. The EDPVR is shifted upwards, however, the overall difference in diastolic stiffness constant between the two groups did not reach statistical significance.

versus 371 ± 23 mg, $p < 0.001$). After normalization for body weight (BW), which was also reduced in the DEX animals, HW/BW was still significantly lower in the DEX animals: (3.62 ± 0.48 *versus* 4.40 ± 0.49 mg/g, $p < 0.001$). Histopathological inspection of the hematoxylin-eosin–stained slides showed reduced wall thickness (1.07 ± 0.10 *versus* 1.40 ± 0.12 mm $p < 0.001$) but no clear signs of cellular necrosis or fibrosis (see Fig. 4).

DISCUSSION

A recent study showed gross histologic abnormalities in myocardial cells of adult rats that had been treated with DEX in the neonatal period (14). These findings are consistent with a preliminary report (15) indicating reduced survival of DEX-treated rats. However, further data on the intermediate and long-term cardiovascular effects of neonatal DEX treatment are lacking. Currently, no human data on intermediate and long-term cardiovascular effects are available. However, substantial cohorts of patients are currently reaching puberty and adulthood. We therefore studied cardiac function in 4-wk-old rats to investigate whether the late effects as described by de Vries *et al.* (14) are preceded by detectable alterations in cardiac function at a younger (prepubertal) age.

We found that rats treated with DEX in the neonatal period have a significantly reduced ventricular wall volume and reduced contractility at 4 wk of age. Cardiac output was maintained in the DEX-treated animals compared with the controls, but end-diastolic volume was increased by 43%, clearly indicating a state of compensatory dilatation. The reduced wall volume and increased cavity volume result in a substantially increased wall stress. Heart rate, diastolic function, and systemic vascular resistance were largely unchanged. We did not normalize our volumetric and contractility parameters for body weight of ventricular weight. However, body weight and ventricular weight were significantly lower in the DEX-treated rats, and therefore the differences between the two groups would be even more pronounced when using normalized parameters.

Myocardial growth in the fetus is obtained by hyperplasia, which changes to hypertrophy after birth. This is shown by an immunohistochemical study in human hearts by Huttenbach *et al.* (26), which reports a comparatively high rate of proliferation from 12 to 28 wk of gestation, with a significant decrease in proliferation after 28 wk of gestation. This finding is in line with stereological studies in fetal and early postnatal human hearts by Mayhew *et al.* (27), which suggest that proliferation ceases approximately 2–3 wk after birth. Thus, DEX treatment in preterm infants occurs during a developmental period in which substantial myocyte hyperplasia would be expected. In rat pups, a rapid switch from myocyte hyperplasia to hypertrophy occurs between postnatal d 3 and 4, as described by Li *et al.* (28). They found that the number of myocytes increased by 68% during the first 3 d and remained constant thereafter, whereas myocyte volume remained relatively constant during the first 3 d and increased 2.5-fold from d 3 to d 12. These studies support that DEX treatment in term rat pups on d 1, 2, and 3 (as performed in our study) corresponds with the clinical DEX treatment of preterm human babies.

With regard to the effects of DEX on ventricular function, several mechanisms may be important. Rudolph *et al.* (29) investigated the effects of cortisol on fetal and neonatal myocardial growth and found that cortisol inhibits myocyte replication. We found a reduced myocardial wall volume that fits with the hypothesis that neonatal DEX treatment leads to an earlier than normal transition from hyperplasia to hypertrophy resulting in fewer myocytes. In addition, DEX treatment alters

Table 2. *Pressure-volume relations: saline-treated (SAL) vs dexamathasone-treated (DEX) rats*

		SAL	DEX	*p* Values
ESPVR	E_{ES} (mm Hg/μL)	2.50 ± 1.39	1.24 ± 0.43	0.028
	ESV_{75} (μL)	34 ± 13	53 ± 20	0.016
dP/dt$_{MAX}$-EDV	S-dP (mm Hg/s/μL)	176 ± 90	102 ± 30	0.032
	dP/dt$_{MAX,70}$ (mm Hg)	12444 ± 4186	8520 ± 3226	0.027
PRSWR	S-PR (mm Hg)	45 ± 13	41 ± 10	NS
	SW_{70} (μL·mm Hg)	2649 ± 560	1915 ± 933	0.050
EDPVR	k (1/μL)	0.017 ± 0.006	0.029 ± 0.026	0.154 (NS)

ESPVR, end-systolic pressure-volume relation; dP/dt$_{MAX}$-EDV, relation between dP/dt$_{MAX}$ and EDV; PRSWR, preload recruitable stroke work relation; EDPVR, end-diastolic pressure-volume relation; E_{ES}, end-systolic elastance; ESV_{75}, volume intercept of ESPVR at 75 mm Hg; S-dP, slope of dP/dt$_{MAX}$-EDV relation; dP/dt$_{MAX,70}$, intercept dP/dt$_{MAX}$-EDV relation at 70 μL; S-PR, slope of PRSWR; SW_{70}, intercept of PRSWR at 70 μL; k, diastolic chamber stiffness constant.
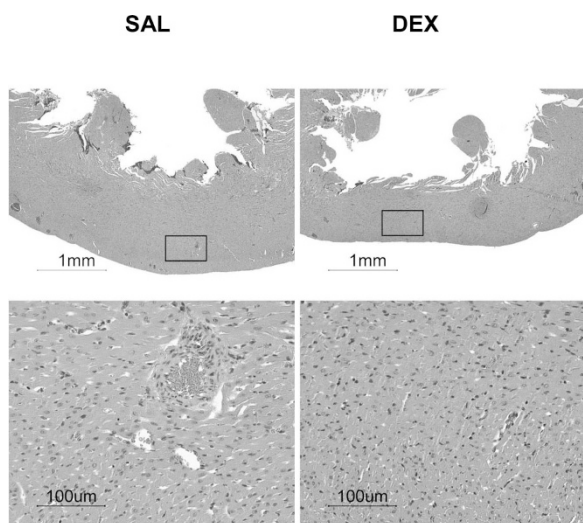
SAL   **DEX**

Figure 4. Hematoxylin-eosin-stained histologic slides. The *top panels* show cross-sections of the LV free wall at the level of the papillary muscles. Note the substantially thinner wall in DEX compared with SAL. The *lower panels* show the myocardium tissue at a higher magnification. No clear signs of cellular necrosis or fibrosis are visible.

the number of steroid receptors in the hypothalamic-pituitary-adrenal axis, which may affect, *e.g.* the renin-angiotensin system, which in time would lead to various cardiovascular disorders including hypertension (30). DEX is also associated with inhibition of angiogenesis and capillary growth (31,32), which may result in relatively poor vascularization. Oxygen supply to the myocardium may then become a limiting factor, especially when the myocardial cells are hypertrophied. This situation may be further aggravated when oxygen demand is enhanced by increased wall stress, as found in our study.

Our results in 4-wk-old rats show a substantial reduction in intrinsic systolic function in the DEX-treated animals; however, cardiac output and pressures are normal. The latter presumably is a result of compensatory dilatation as evident from the significantly increased ventricular volumes, indicating that the animals invoke their Starling mechanism. Combined with the reduced wall volume, these alterations markedly increased wall stress. The rightward shifts of both the ESPVR, evidenced by its increased volume intercept ESV_{75}, and the EDPVR indicate a process of remodeling. The finding that end-diastolic pressures and stiffness were relatively normal despite an increased end-diastolic volume suggests structural changes such as slippage of cardiomyocytes (33). We speculate

that excessive hypertrophy initially fully compensates, but in a later stage this mechanism may fail and lead to a state of compensatory dilation (as found in our prepubertal rats) and eventually may progress to cardiac failure, as suggested by the study of de Vries *et al.* (14).

The pressure-volume loops in our study were obtained with a miniature conductance catheter. This methodology has been extensively validated in various species, including mice, which are substantially smaller than 4-wk-old rats (17–19). However, the experience with the conductance catheter applied in young rats is limited. Therefore, to support our main findings, we also performed echocardiographic measurements in a small group of additional animals. The echocardiographic findings were fully consistent with the hemodynamic data obtained by the conductance catheter method.

Whether our findings are applicable to humans is unknown. At this point no cardiac abnormalities have been reported in children in the age group (prepuberty, 10–12 y old) corresponding to the 4-wk old rats in our study. However, from the lack of such reports we would not draw the conclusion that findings similar to those found in our rats can be excluded in humans. First, widespread routine DEX treatment started in the early 1990s, therefore the number of children that have reached puberty is still fairly limited. Second, the effects in this age group may be subclinical (as suggested by our findings in rats), and therefore such studies may not have seemed to be warranted. This may explain that, to our knowledge, no studies are available regarding the long-term effects of DEX on cardiac function (or cardiac dimensions) in humans. In contrast to possible late side effects, the early cardiac adverse effects of neonatal DEX treatment have been well studied in human babies. Serial echocardiographical measurements have documented a transient phase of LV hypertrophy that starts during treatment and is generally resolved 2–3 wk after DEX weaning (12,13,34,35). The same phenomenon of transient early hypertrophy has also been shown to be present in the rat model (11). This finding supports the validity of the rat model and the treatment dose. The rat model has also been used extensively in neurologic studies, and these studies have demonstrated important late side effects of DEX treatment (6). Importantly, recent clinical studies indicate very similar findings in humans (5,8,9,36), again showing the correspondence with the rat model.

The DEX treatment in our study was based on extrapolation of the human treatment protocol. The 3-d duration of the DEX

exposure represents about 15% of the lactation period in the rat (21 d), which corresponds with a typical 21-d treatment period in humans. Similar to the typical clinical treatment, a tapering dose of 0.5, 0.3, and 0.1 μg/g was used. The same approach was used in most previous cardiovascular and neurologic studies in the rat.

Obviously, extrapolation of our data to humans must be done with extreme caution, but we feel that further hemodynamic studies in humans should be considered. Screening of children previously treated with DEX may be relevant to initiate early medical intervention if necessary. It is important to realize that in our study alterations in systolic function were derived from pressure-volume relations, which are difficult to obtain in clinical practice. Our data regarding ejection fraction and cardiac output, systolic, and diastolic pressures, which are more commonly used in patient studies, did not reveal abnormal function. Therefore, determination of absolute cardiac volumes, which were found to be enlarged, presumably as a compensatory mechanism, might be a realistic and useful diagnostic possibility. In addition, recently developed echocardiographic methods like tissue-Doppler myocardial velocity imaging or strain rate imaging may prove to be of value.

Limitations. We used term male rat pups as a model for premature human babies. This model has been proven useful for neurologic studies because, with regard to brain development, a 7-d-old rat is roughly equivalent to a full-term human infant and, consequently, term rat pups may correspond well to premature infants (37). Furthermore, at d 1–3 after birth, the rat myocardium still shows substantial hyperplasia (28), similar to the preterm human myocardium (26,27). Thus, with respect to the transition from myocyte hyperplasia to hypertrophy, the term rat pub appears to be a good model for the preterm human infant. However, potential differences in the degree of maturation of neurohormonal systems, receptor mechanisms, and innervation (38) may also be influential in the response to DEX treatment. The development of these systems has been studied in the rat (39), but very few data are available for the (preterm) human situation. Thus, species differences may be present, but other studies (11,14,40,41) have used the same model and shown that several of the effects of DEX treatment found in humans, including early transient hypertrophy, were also found in the rat model.

We used only male pups to limit the within-group variability of the various parameters, but potentially gender-specific responses could be present. Neurologic studies appear to indicate that males are more susceptible to adverse effects of neonatal glucocorticoid treatment, however, we are not aware of studies showing gender-specific effects on the cardiovascular system.

The relatively low ejection fraction and mean arterial pressure in the control (SAL) animals indicate that cardiac function was somewhat depressed, presumably as a result of general anesthesia. We cannot rule out that the DEX animals are more sensitive to our anesthesia, resulting in relatively more depressed cardiac function. However, by using FFM anesthesia we aimed at minimizing this effect (42). Furthermore, our additional echocardiographic measurements were obtained during isoflurane inhalation and indicated very similar differences between DEX and SAL treated rats as the invasive data.

In conclusion, neonatal DEX treatment in rats leads to unfavorable changes in systolic left ventricular function in the prepubertal period. Whether this represents an early stage of heart failure remains to be demonstrated. Further studies are required to investigate the long-term cardiac effects of DEX treatment and the underlying mechanisms. If applicable to humans, a rather large patient population is involved and early cardiac screening of children treated with DEX may be indicated.

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