Neonatal glucocorticoid overexposure alters cardiovascular function in young adult horses in a sex-linked manner

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Abstract

Prenatal glucocorticoid overexposure has been shown to programme adult cardiovascular function in a range of species, but much less is known about the long-term effects of neonatal glucocorticoid overexposure. In horses, prenatal maturation of the hypothalamus–pituitary–adrenal axis and the normal prepartum surge in fetal cortisol occur late in gestation compared to other precocious species. Cortisol levels continue to rise in the hours after birth of full-term foals and increase further in the subsequent days in premature, dysmature and maladapted foals. Thus, this study examined the adult cardiovascular consequences of neonatal cortisol overexposure induced by adrenocorticotropic hormone administration to full-term male and female pony foals. After catheterisation at 2–3 years of age, basal arterial blood pressures (BP) and heart rate were measured together with the responses to phenylephrine (PE) and sodium nitroprusside (SNP). These data were used to assess cardiac baroreflex sensitivity. Neonatal cortisol overexposure reduced both the pressor and bradycardic responses to PE in the young adult males, but not females. It also enhanced the initial hypotensive response to SNP, slowed recovery of BP after infusion and reduced the gain of the cardiac baroreflex in the females, but not males. Basal diastolic pressure and cardiac baroreflex sensitivity also differed with sex, irrespective of neonatal treatment. The results show that there is a window of susceptibility for glucocorticoid programming during the immediate neonatal period that alters cardiovascular function in young adult horses in a sex-linked manner.

Introduction

Human epidemiological observations and studies in experimental animals have demonstrated that environmental conditions during pregnancy that alter birth weight can have consequences for offspring phenotype long after birth.[1–4] More specifically, changes in maternal nutrition and levels of stress during pregnancy lead to cardiovascular, metabolic and endocrine dysfunction in the adult offspring in a range of laboratory and farm species including rodents, primates, pigs and sheep.[5] In horses, maternal undernutrition and dietary manipulations in late pregnancy are known to alter endocrine and metabolic function in newborn and juvenile foals in association with reduced birthweight.[6–8] Similarly, manipulating intrauterine growth by embryo transfer between horse breeds of different sizes leads to specific changes in baroreceptor sensitivity, glucose metabolism, insulin sensitivity and adrenal function in newborn foals or yearlings, both when birth weight is restricted or enhanced with respect to their genetic norms.[9–12] Even when there is little if any change in birth weight, adult cardiometabolic phenotype can be programmed by environmental cues acting during intrauterine development.[5]

Many of the environmental conditions known to programme phenotype *in utero* raise circulating glucocorticoid levels in the mother and/or fetus.[13,14] Indeed, this glucocorticoid overexposure may contribute to the developmental programming, as glucocorticoids are known to regulate fetal growth and development both directly and indirectly by changes in placental function.[14,15] In addition, exposure of the offspring to synthetic glucocorticoids *in utero* by maternal administration during pregnancy has been shown to induce postnatal cardiometabolic and endocrine dysfunction in the offspring of several species.[16–19] In pregnant mares for instance, maternal administration of dexamethasone in late pregnancy is known to alter pancreatic β-cell function in their 12-week-old foals in the absence of any change in birth weight.[20]

Furthermore, in rats, neonatal administration of the synthetic glucocorticoid, dexamethasone, programmes cardiac dysfunction in the adult offspring.[21,22] However, much less is known about the long-term cardiovascular implications of overexposure to the natural glucocorticoids, particularly in the immediate neonatal period.[17,23]

In horses, maturation of the fetal hypothalamic–pituitary–adrenal (HPA) axis occurs relatively late in gestation with plasma cortisol levels continuing to rise in the normal full-term foal in the hours after birth in contrast in findings in other precocious species.[24] Cortisol levels rise still further in the days after birth in foals that are premature or dysmature at birth or that develop maladaptation syndrome after birth.[25,26] Consequently, the period of programming by endogenous glucocorticoid is likely to extend into the neonatal period in the horse, as seen in more altricial species like the rat.[10] Indeed, recent studies have shown the elevating cortisol levels experimentally in normal foals in the first few days after birth affects functioning of the HPA axis and pancreatic β cells 2–12 weeks later and HPA axis function and muscle insulin receptor abundance in the young adult horse.[27–30] However, little is known about the effects of neonatal overexposure to glucocorticoids on equine cardiovascular function later in life, although terminal differentiation of several tissues, like the lung and kidney, is known to continue after birth in the foal, unlike many other precocious species.[31] Indeed, blood pressure (BP) and baroreceptor sensitivity continue to alter developmentally during the first few weeks of life in healthy-term foals.[32–34] This study therefore examined the effect of raising endogenous cortisol concentrations in newborn pony foals by adrenocorticotropic hormone (ACTH) administration on their cardiovascular function and baroreceptor sensitivity 2–3 years later relative to controls that received saline. It also investigated the extent to which the long-term cardiovascular effects of neonatal cortisol overexposure were sex-linked, as metabolic function has been shown to be sexually dimorphic in both newborn and older ponies.[28,34] The study tested the hypothesis that cardiovascular responsiveness and baroreceptor sensitivity in young adult ponies are altered by neonatal cortisol overexposure.

Materials and methods

Animals

Seventeen ponies (nine female and eight males) were born spontaneously at full term (approximately 330 d in pony mares) and weaned at 5–6 months. Thereafter, until catheterisation, they were kept in single sex groups at grazing during the day and in covered yards at night with *ad libitum* access to hay and water. Body condition score was maintained at moderate from weaning to the end of the protocol. All animals received equine tetanus antitoxin shortly after birth and regular worming and hoof trimming. After catheterisation as adults, the animals were housed individually in stables within sight and sound of other horses and with *ad libitum* hay and water.

Experimental procedures

All procedures were carried out under the Animal (Scientific Procedures) Act 1986 of the UK Government and were approved by the Animal Welfare and Ethical Review Body of the University of Cambridge

Foals

After birth, foals received intramuscular injections of either saline as a control procedure (0.9% NaCl im; $n = 8$, four males and four females) or long-acting $ACTH_{1-24}$ (0.125 mg im; $n = 9$, four males and five females, Depot Synacthen; Alliance Pharmaceuticals Ltd, Wiltshire, UK) to raise plasma cortisol levels to values similar to those seen in premature, dysmature or ill foals.[26,35,36] The injections were given twice daily at 09.00 h and 17.00 h for the first 5 d after birth. Blood samples were taken daily from the jugular vein during this period to measure plasma cortisol concentrations using an immunoassay validated for equine plasma as described previously.[27] Over the 5 d of treatment, plasma cortisol concentrations were significantly higher in ACTH treated (183.7 ± 22.5 ng/ml, $n = 9$) than control foals (20.3 ± 2.4 ng/ml, $n = 8$, $P < 0.01$) and were unaffected by sex of the foals in either group ($P > 0.05$, two-way analysis of variance (ANOVA)). Foals were assigned to either the saline or ACTH group in the order in which they were born on the basis of their sex to ensure an even sex distribution between treatments. Foal birth weight did not differ with sex or between the treatment groups to which they were assigned (Table 1, $P > 0.05$, two-way ANOVA).

Adults

Surgical procedures. Between 23 and 34 months of age, the ponies were catheterised under strict aseptic conditions after an overnight fast. Anaesthesia was induced using ketamine (2.2 mg/kg, Ketaset; Ford Dodge Animal Health Ltd, Southampton, UK) and diazepam (0.01 mg/kg Diazepam; Hameln Pharmaceuticals Ltd, Gloucester, UK) given via the jugular vein. After intubation, anaesthesia was maintained with 1.5–2.0% isoflurane in O_2, using intermittent positive pressure ventilation. The horses were placed in left lateral recumbency on an inflatable operating table. Polyvinyl catheters (outer diameter 1.52 mm; inner diameter 0.86 mm; Critchley, Electrical Products Ltd, Silverwater, New South Wales, Australia) were inserted into the circumflex artery and vein, with their tips advanced into the dorsal aorta and vena cava, respectively. The catheters were filled with heparinised saline (100 IU heparin/ml in 0.9% w/v NaCl) and sealed with sterile brass pins. They were exteriorised via a keyhole incision in the flank and housed in a bag sutured to the skin. Antibiotics (1 ml/25 kg, procaine penicillin BP 200 mg and dihydrostreptomycin sulphate BP (vet) 250 mg; Pen & Strep; Norbrook Laboratories Ltd, UK) and an anti-inflammatory (1.1 mg/kg, flunixin meglumine; Finadyne 50 mg; Shering – Plough Ltd, Wewyn Garden City, Herts, AL7 1TW, UK) were given intramuscularly at the end of the surgery. Catheters were flushed daily with heparinised saline (100 IU heparin/ml in 0.9% w/v NaCl) before cardiovascular studies were started after at least 5 d of post-operative recovery. All animals had been catheterised previously as yearlings for other metabolic and endocrine studies.[28,30]

Cardiovascular studies. Measurements of arterial BP were made via a pressure transducer (COBE; Argon Division, Maxxim Medical, Athens, TX, USA) set at the level of the heart using a custom-built data acquisition system (Maastricht-Programmable AcQuistion – IDEEQ 2.05, Maastricht Instruments, Maastricht, The Netherlands). Diastolic, systolic and mean arterial BPs (mmHg) together with heart rate (HR, beats per minutes) were obtained from the pressure recordings.[37,38] After 10 min of continuous recording of basal BPs and HR, either phenylephrine (PE, 6 µg/kg/min; Sigma-Aldrich Co. Ltd, Haverhill, UK) or sodium nitroprusside (SNP, 2.5 µg/kg/min; Sigma-Aldrich Co. Ltd, Haverhill, UK) was infused intravenously for 10 min to induce episodes of acute hypertension or acute hypotension, respectively. Recording was continued throughout the infusion period and

Table 1. Mean ± SEM body weight at birth and at the time of together with basal values of arterial diastolic, systolic and mean arterial BP (mmHg) and HR (beats per min, bpm) before infusion of PE and SNP and the maximum and minimum values of these variables during the 10 min of infusion in all ponies at 2–3 years of age and in the males and females separately after neonatal treatment with either saline or ACTH. Doses of drugs are given in the text

	Saline			ACTH		
	All	Male	Female	All	Male	Female
Number of ponies	8	4	4	9	4	5
Body weight						
At birth (kg)	28.6 ± 2.6	31.2 ± 4.6	26.1 ± 2.6	28.5 ± 2.3	29.2 ± 4.8	27.9 ± 2.1
At study (kg)	230 ± 16	238 ± 14	223 ± 32	246 ± 14	248 ± 28	244 ± 17
Basal values						
Diastolic BP (mmHg)*	89.3 ± 4.3	99.6 ± 3.7	79.1 ± 1.3	92.7 ± 4.3	100.8 ± 5.7	86.3 ± 4.7
Systolic BP (mmHg)	135.2 ± 8.3	143.6 ± 5.6	126.8 ± 15.6	139.7 ± 9.3	154.5 ± 14.1	127.9 ± 10.6
Mean BP (mmHg)	104.6 ± 4.8	114.3 ± 3.6	95.0 ± 5.6	108.4 ± 5.8	118.7 ± 8.3	100.2 ± 6.4
HR (bpm)	46.3 ± 2.1	48.0 ± 3.5	44.7 ± 2.4	45.9 ± 1.8	42.6 ± 1.8	48.7 ± 2.3
PE infusion						
Maximum diastolic BP (mmHg)	167.6 ± 9.7	177.8 ± 11.3	157.3 ± 15.6	159.0 ± 7.0	168.8 ± 12.2	151.2 ± 7.1
Maximum systolic BP (mmHg)	211.9 ± 13.9	217.2 ± 6.0	206.5 ± 29.0	217.7 ± 15.9	236.2 ± 25.8	203.0 ± 19.6
Maximum mean BP (mmHg)	180.6 ± 10.6	189.7 ± 9.5	171.5 ± 19.4	177.4 ± 9.6	190.3 ± 16.0	167.1 ± 10.8
Minimum HR (bpm)	28.0 ± 1.4	27.6 ± 1.7	28.4 ± 2.5	28.2 ± 1.5	28.4 ± 2.5	28.9 ± 2.5
SNP infusion						
Minimum diastolic BP (mmHg)*	61.7 ± 4.9	71.6 ± 4.5	52.0 ± 5.0	69.1 ± 4.9	82.5 ± 2.5	58.4 ± 4.4
Minimum systolic BP (mmHg)*	97.4 ± 6.7	108.4 ± 8.7	86.4 ± 7.2	104.8 ± 8.5	120.9 ± 9.4	91.9 ± 10.5
Minimum mean BP (mmHg)*	73.3 ± 5.5	85.0 ± 5.9	61.6 ± 4.2	81.4 ± 5.8	94.9 ± 4.5	70.5 ± 6.6
Maximum HR (bpm)	79.1 ± 4.9	74.3 ± 5.9	83.9 ± 7.9	76.2 ± 3.8	76.7 ± 8.5	75.7 ± 3.1

*Sex effect by two-way ANOVA $P < 0.01$.

for 10 min after cessation of infusion. The two infusions were carried out on consecutive days in a random order. The mean age of the ponies at the time of the cardiovascular study was 30.9 ± 0.8 months and did not differ with sex or neonatal treatment ($n = 17$, $P > 0.05$, two-way ANOVA). Body weight at the time of the cardiovascular studies also did not differ with sex or neonatal treatment (Table 1).

All animals were familiar with the environment used for the experimental studies as they had undergone additional metabolic and endocrine assessments in the preceding month. At the time of the cardiovascular measurements, none of the females showed any of the standard behavioural signs of estrus, such as tail deviation, rhythmic eversion of the clitoris, frequent urination, pelvic lowering or straddling of the hind limbs in the vicinity of a male.[39] At the end of the cardiovascular protocol, the animals were either rehomed ($n = 9$) after discharge from the Animals (Scientific Procedures) Act 1986 or euthanised ($n = 8$) by intravenous administration of a lethal dose of anaesthetic (Pentobaribitone sodium, 200 mg/kg, Pentoject; Animal Care Ltd, Dunnington, York, UK) for the collection of tissues for teaching and other research studies.

Data analyses

For each animal, the average for mean arterial BP (mean BP) and HR were calculated every 10 s from the 500-Hz recordings during the 10-min baseline, infusion and recovery periods. Cardiac baroreflex curves were then constructed by plotting values for mean BP against HR during basal conditions and during changes induced by infusion of PE and SNP. The correlation data were fitted by a logistic sigmoidal curve and the maximum slope of the relationship, representing the gain of the cardiac baroreflex was calculated as ((HRmax − HRmin) × Gain coefficient)/4), as described previously.[40] Mean cardiac baroreflex curves were then constructed for each treatment group with SEM values for both mean BP and HR. Curves through the mean data points were drawn using a Fit spine/LOWESS (locally weighted scatterplot smoothing) function.[38]

To further evaluate the pressor and cardiac chronotropic responses to each agonist, mean BP and HR responses to PE or SNP were also constructed by plotting the maximal change in value from the mean baseline for each minute of infusion for each animal. Changes from baseline in continuous cardiovascular data were then summarised over 5-min epochs and used to calculate the areas either under the curve (AUC) or above the curve (AAC) for the changes in mean BP and HR from baseline. Data from these 5-min epochs were then averaged for all the animals in each treatment group to provide mean pressor and cardiac chronotropic responses to the agonists and are presented with their respective SEM.[38]

Statistical analyses

All data are expressed as mean ± SEM and analysed statistically by two-way ANOVA using neonatal treatment and time or neonatal treatment and sex of the ponies as factors with the Holm–Sidak *post hoc* test. Statistical analyses were performed using

Sigma-Stat (Statistical Software version 2.0, San Jose, CA, USA). For all statistical tests, significance was accepted when $P \leq 0.05$.

Results

Basal cardiovascular measurements

During the basal recording period before infusion of PE or SNP, there were no significant differences in the average systolic, diastolic or mean arterial BP between the ACTH- and saline-treated groups of ponies (Table 1). However, diastolic pressure differed with sex of the ponies and was higher in males relative to females, irrespective of their neonatal treatment (Table 1, two-way ANOVA, $n = 17$, $P < 0.01$). Neither systolic nor mean BP differed with sex of the ponies during the basal recording period (Table 1). There were also no significant differences in basal HR with either sex or neonatal treatment (Table 1). These basal HR and BP measurements were within the range of values published previously for adult ponies and other small equine breeds using non-invasive methods or short-term percutaneous catheterisation.[41–45]

Hypertensive challenge: cardiovascular responses to PE

There were rapid increases in mean BP and reductions in HR during the first 5 min of PE infusion in all the ponies, irrespective of their neonatal treatment. These data are presented for all animals in each treatment group irrespective of sex (Fig. 1a – i and ii) and for the males and females separately (Fig. 1b and c, respectively). The maximum values of the absolute and increment in mean BP during PE infusion were not affected by sex of the animals or their neonatal treatment (Table 1). These values for diastolic and systolic BP as well as the lowest HR and the maximum decrement in HR during infusion were also unrelated to sex or neonatal treatment (data not shown, two-way ANOVA, $P > 0.05$, all cases).

When the sexes were combined within each treatment group, neonatal treatment had a significant effect on the AUC for the change in mean BP during and after the PE infusion (Fig. 1a – iii). *Post hoc* analyses showed that the AUC for mean BP was significantly reduced in the ACTH relative to the saline-treated ponies for the first and second 5-min epochs of infusion (Fig. 1a – iii). However, analysis of the data by sex showed that the depressive effect of neonatal treatment on the AUC for the change in mean BP during infusion was due primarily to the males (Fig. 1b – iii) and not the females (Fig. 1c – iii). There were no effects of neonatal treatment on the AAC for the change in HR for either 5-min period during infusion, when the sexes were combined (Fig. 1a – iv). However, in the males only, neonatal treatment reduced the AAC for the decrement in HR during the 5-min epochs during PE infusion (Fig. 1b – iv and c – iv). Neonatal treatment also had no significant effect on the AAC for the change in HR during the recovery period either when the sexes were combined or analysed separately (Fig. 1).

Hypotensive challenge: cardiovascular responses to SNP

During SNP infusion, there were gradual falls in mean BP and increases in HR during the first 5 min of infusion in all the animals studied (Fig. 2). When the data from the ACTH- and saline-treated groups were analysed with the sexes combined within each group, neonatal treatment had no effect on the fall in mean BP or on the rise in HR during infusion (Fig. 2a – i and ii). The minimum mean BP and the maximum change in mean BP from baseline during the 10-min infusion period were also unaffected by neonatal treatment (Table 1). Similar findings were observed for the minimum diastolic and systolic BPs and their mean decrements during infusion (data not shown, two-way ANOVA, $P > 0.05$, all cases). With the sexes combined, neonatal treatment affected the AAC for the change in mean BP in response to SNP, which *post hoc* analyses showed was due primarily to a slower recovery of mean BP post-infusion, with a greater AAC in ACTH than saline-treated ponies during both 5-min periods after ending the infusion (Fig. 2a – i and iii). There were no differences in the HR responses, nor in AUC for the change in HR, with treatment when the sexes within each group were combined (Table 1; Fig. 2a – ii and iv).

Analysis of data by sex showed that males and females differed in their BP responses to SNP infusion, particularly after neonatal ACTH treatment (Fig. 2b and c). Minimum diastolic, systolic and mean BPs in response to SNP infusion were lower in females than males irrespective of treatment, in line with the lower basal diastolic BP (Table 1). For the first 5 min of infusion, the AAC for mean BP was significantly greater in ACTH than saline-treated females but not males (Fig. 2b – iii and c – iii). The slow recovery of mean BP after ending the infusion in the ACTH-treated animals was also more pronounced in females than males, with a significantly greater AAC for mean BP for the two 5-min periods after ending infusion in the female but not the male ACTH-treated ponies relative to their saline-treated counterparts (Fig. 2b – iii and c – iii). No significant differences in the HR responses were observed between the sexes either during or after ending SNP infusion (Table 2; Fig. 2b – ii and iv, and c – ii and iv).

Cardiac baroreflex curves

The changes in HR in response to alterations in mean BP induced by both infusions were used to generate cardiac baroreflex function curves (Fig. 3). There were no differences in the minimum or maximum HR, or in the baroreflex gain, between the saline- and ACTH-treated ponies when the two sexes in each treatment group were combined (Table 2; Fig. 3). However, there were interactions between neonatal treatment and the sex of the ponies in determining the gain of the baroreflex curve with differences between males and females in the control group and with neonatal treatment in female but not male ponies (Table 2). The overall gain of the autonomic baroreflex function was markedly blunted in male relative to female ponies in the control, saline-treated groups (Table 2). Conversely, neonatal ACTH treatment reduced the gain of the autonomic baroreflex function only in female but not male ponies (Table 2).

Differential analysis of the changes in HR in response to acute hypotension and acute hypertension can be used to give further insight to the partial effects on the sympathetic and parasympathetic components of the baroreflex function curve.[32,37] This analysis suggested that sympathetic dominance was affected by both neonatal treatment and the sex of the ponies (Fig. 4a). Within the saline-treated ponies, the sympathetic component of the baroreflex function curve seemed more dominant in females than males with a faster increment in HR in response to acute hypotension in the females (Fig. 4a – ii and iii). Neonatal ACTH treatment had no apparent effect on the sympathetic component of the baroreflex function curve in the males (Fig. 4a – ii) but attenuated the HR response to acute hypotension in the females (Fig. 4a – iii).
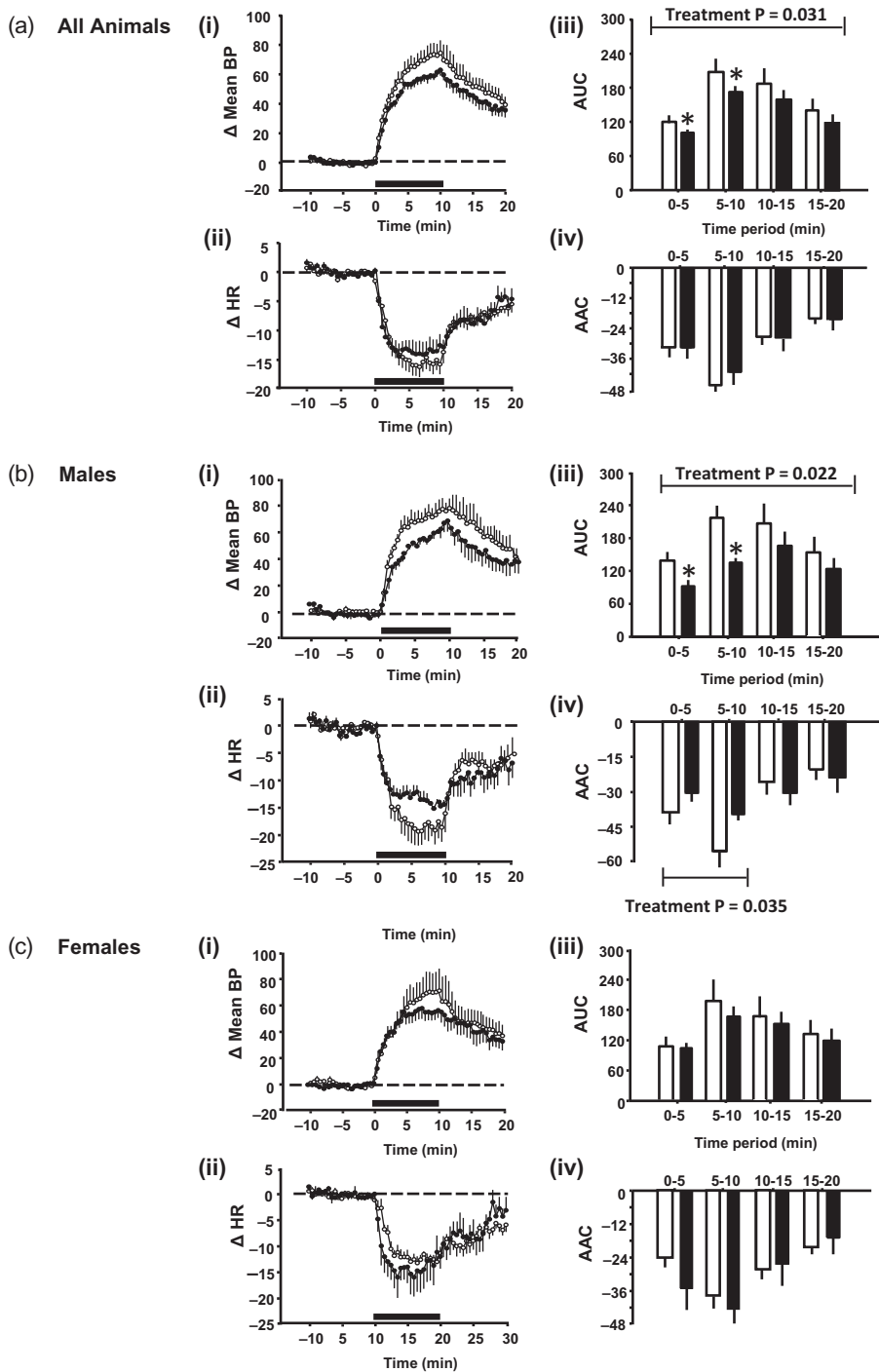
Fig. 1. Mean (±SEM) values of the change (Δ) in mean arterial BP (mmHg, i) and HR (beats per minute, bpm, ii) from baseline with respect to time from the start of infusion and the AUC (units = mmHg/5 min × 10^{-2}) for mean arterial BP (iii) and AAC (units = bpm/5 min × 10^{-2}) for HR (iv) over 5-min periods in response to intravenous infusion of PE for 10 min (bar, 6 µg/kg/min) in ponies at 2–3 years of age after neonatal treatment with either saline (open symbols and bars, n = 8) or ACTH (filled symbols and bars, n = 9) for (a) all animals irrespective of sex, (b) males only (saline, n = 4; ACTH, n = 4) and (c) females only (saline, n = 4; ACTH, n = 5). Significant treatment effect (two-way ANOVA shown with a bar with the Holm–Sidak *post hoc* test shown with *, $P < 0.05$, for specific time periods).

Neither neonatal treatment nor sex of the ponies appeared to affect the parasympathetic component of the autonomic baroreflex function (Fig. 4b).

Discussion

The data show that neonatal overexposure to cortisol induced by ACTH treatment in the days immediately after birth programmes long-term changes in cardiovascular function and cardiac baroreceptor sensitivity in a sex-linked manner in young adult horses. More specifically, neonatal cortisol overexposure reduced the pressor response to PE in the male but not female ponies.

Conversely, in females but not males, neonatal cortisol exposure enhanced the early hypotensive response to SNP and slowed recovery of mean BP to normal values after the end of infusion without affecting the HR response. This means that for a greater SNP-induced fall in mean BP in the ACTH-treated females, there was a similar HR increment to that seen in the saline-treated controls. Further analysis of these data suggested a blunted sympathetic component to the cardiac autonomic baroreflex function in the females after neonatal cortisol overexposure that was not seen in the ACTH-treated males. However, relative to the females, males had a depressed gain in autonomic baroreflex function in the control group and a higher basal diastolic pressure, irrespective of
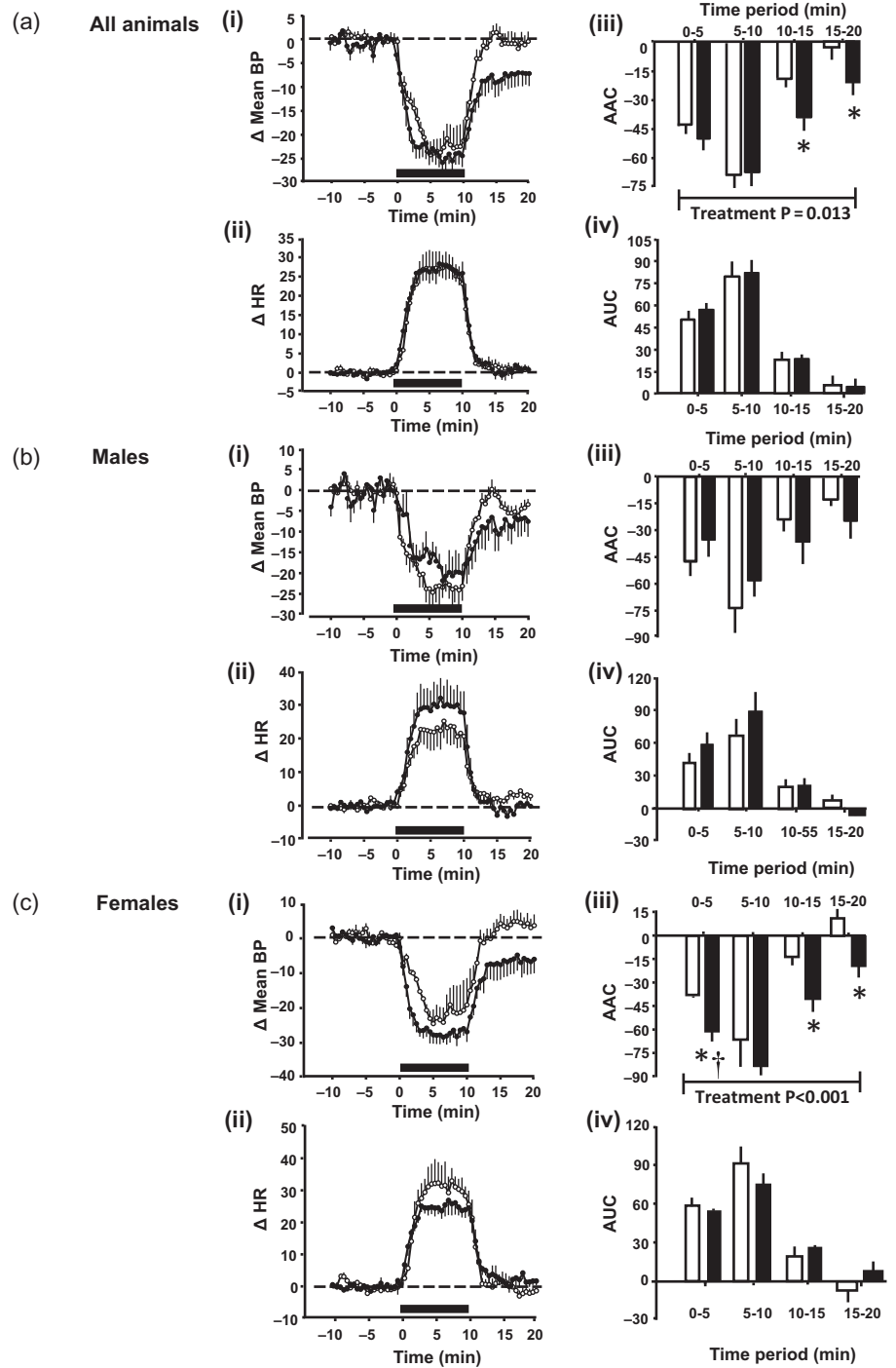
Fig. 2. Mean (± SEM) values of the change in arterial BP (mmHg, i) and HR (beats per minute, bpm, ii) from baseline with respect to time from the start of infusion and the AAC (units = mmHg/5 min × 10⁻²) for mean arterial BP (iii) and AUC (units = bpm/min/5 min × 10⁻²) for HR (iv) over 5-min periods in response to intravenous infusion of SNP for 10 min (bar, 2.5 µg/kg/min) in ponies at 2–3 years of age after neonatal treatment with either saline (open symbols and bars, $n = 8$) or ACTH (filled symbols and bars, $n = 9$) for (a) all animals irrespective of sex, (b) males only (saline, $n = 4$; ACTH, $n = 4$) and (c) females only (saline, $n = 4$; ACTH, $n = 5$). Significant treatment effect (two-way ANOVA by shown with a bar with the Holm–Sidak *post hoc* test shown with a *, $P < 0.05$ for specific times periods). †Significantly different from value in the males at the same time period (two-way ANOVA, $P < 0.02$ with the Holm–Sidak *post hoc* test).

neonatal treatment. Collectively, these findings show that neonatal cortisol overexposure, like that seen in premature and dysmature foals, has long-term consequences for cardiovascular function in the horse in support of the study hypothesis. However, the basal cardiovascular profile and the specific changes in adult cardiovascular function programmed by neonatal ACTH treatment depend on the sex of the pony.

Previous studies in a range of species have shown that prenatal overexposure to either synthetic or natural glucocorticoids affects development of the heart and blood vessels and leads to postnatal cardiovascular dysfunction with hypertension and altered baroreceptor function in adulthood.[17–19,46–49] The current study in ponies shows that long-term cardiovascular function and its regulation are also affected in early adulthood by overexposure to the natural glucocorticoid, cortisol, in the immediate neonatal period. The smaller hypertensive response to α₁-adrenergic receptor agonist, PE, and the slower recovery of BP after cessation of infusion of the nitric oxide (NO) donor, SNP, despite normal HR responses, indicate that the regulation of peripheral vascular tone may be impaired in young adult horses after neonatal cortisol overexposure. Specifically, the data suggest impaired α₁-adrenergic constrictor and/or enhanced NO-dependent dilator function in the peripheral vasculature of horses treated with ACTH during the neonatal period. In premature human infants treated neonatally

Table 2. Maximum (Max) increments and decrements in mean BP (mmHg) and HR (beats per minute, bpm) from basal values during the 10-min infusion of PE and SNP in all ponies at 2–3 years of age and in the males and females separately after neonatal treatment with saline or ACTH. Doses of drugs are given in the text

	Saline			ACTH		
	All	Male	Female	All	Male	Female
Number of ponies	8	4	4	9	4	5
PE infusion						
Max increment in mean BP (mmHg)	76.4 ± 8.5	80.2 ± 8.4	72.8 ± 16.0	66.8 ± 5.1	72.2 ± 7.5	62.6 ± 7.0
Max decrement in HR (bpm)	18.3 ± 1.8	21.6 ± 2.7	14.9 ± 0.89	17.3 ± 1.9	15.9 ± 0.4	18.5 ± 3.4
SNP infusion						
Max decrement in mean BP (mmHg)	28.2 ± 3.9	26.8 ± 4.4	29.7 ± 7.0	28.1 ± 2.4	24.5 ± 4.3	30.9 ± 2.3
Max increment in HR (bpm)	32.6 ± 4.2	27.2 ± 5.5	38.0 ± 5.7	31.8 ± 3.5	34.5 ± 6.6	29.7 ± 3.8
Gain of baroreflex curve* (bpm/mmHg)	1.07 ± 0.28	0.55 ± 0.18†	1.60 ± 0.57	0.69 ± 0.14	0.88 ± 0.28	0.54 ± 0.08†

*Significant interaction between sex and neonatal treatment (two-way ANOVA $P < 0.01$, significant effect of sex within saline-treated group and significant effect of treatment within females)
†Significantly less than the value in the saline-treated females ($P < 0.01$ two-way ANOVA with the Holm–Sidak *post hoc* test.
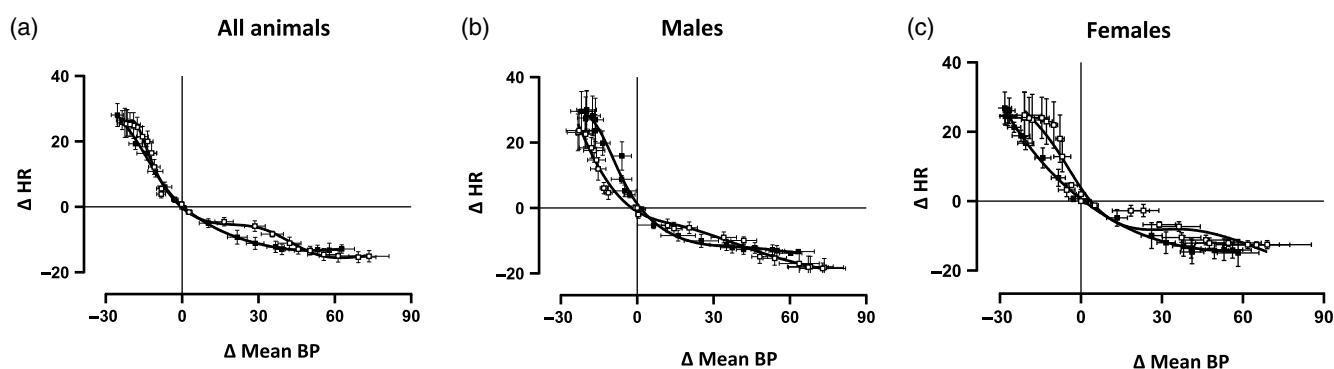
Fig. 3. Baroreflex function curves showing the relationship of mean arterial BP (mmHg) and HR (beats per minute) (mean ± SEM on x and y axes) in response to a hypertensive challenge (PE infusion PE: 6 µg/kg/min) and a hypotensive challenge (SNP: 2.5 µg/kg/min) in ponies at 2–3 years of age after neonatal treatment with either saline (open symbols, $n = 8$) and ACTH (filled symbols, $n = 9$) for (a) all animal irrespective of sex, (b) males only (saline, $n = 4$; ACTH, $n = 4$) and (c) females only (saline, $n = 4$; ACTH, $n = 5$).

with dexamethasone, cardiovascular responses to psychological stress were blunted at school age with smaller increases in plasma norepinephrine.[50] Furthermore, in sheep, maternal antenatal treatment with dexamethasone attenuated vascular vasoconstrictor responses to noradrenaline in the newborn but not adult offspring.[51–53] This treatment also enhanced the vasodilator response of femoral vessels to SNP and the vasoconstrictor response to blockade of NO production in the newborn lamb.[51,53] Similarly, antenatal treatment of pregnant sheep with the synthetic glucocorticoid, betamethasone, programmed an enhanced dilator response to the endothelium-dependent agonist, acetylcholine in small resistance arteries of their 1- to 2-year-old offspring.[54] Consequently, altered vascular NO production and/or NO sensitivity may also contribute to the impaired pressor responses to PE and the prolonged depressor effect of SNP observed in the current study of adult horses overexposed to cortisol neonatally.

The cortisol-induced changes in the BP responses in the present study may also reflect programmed cardiac dysfunction affecting stroke volume and cardiac output. Neonatal treatment of term rat pups with dexamethasone led to thinning of the left ventricular wall and to decreased proliferation and accelerated terminal differentiation of the cardiomyocytes by weaning in association with modifications in cardiac DNA methylation.[55–57] In adulthood, this neonatal treatment led to a reduced heart weight, hypertrophic

cardiomyopathy, hypertension and a shorter life span.[21,22,58–60] Functionally, the hearts of adult rats treated with dexamethasone during the neonatal period had an elevated left ventricular end-diastolic pressure, a smaller ejection fraction and did not adapt to imposed changes in pre- and after-load, indicative of a failed Frank–Starling mechanism.[21,22] These impaired cardiac responses were accompanied by lower circulating levels of NO metabolites and reduced cardiac abundance of several sodium transporters.[22,61] Stroke volume is also reduced in response to stress in school-aged children treated with dexamethasone neonatally for prematurity.[50]

Baroreceptor set point and sensitivity are known to change perinatally in foals and lambs to accommodate the rising postnatal BP.[32,37,62] There is a rightward shift in the baroreflex function curve with increasing postal age in both species, which is accompanied by alterations in the relative contribution of the vagal and sympathetic components of HR regulation towards increased sympathetic dominance, particularly in the foal.[32,62] However, by adulthood, parasympathetic activity appears to be the predominant factor in the response to increasing BP in horses.[63] Treatment of pregnant ewes with synthetic glucocorticoids has been shown to cause a rightward shift in the baroreflex function curve in the offspring during fetal, neonatal, pre-weaning juvenile and adult life.[51,52,64–66] It also attenuated the gain of the baroreflex from as early as 6 weeks of postnatal life.[52,65,66] These alterations in

(a) Sympathetic dominance
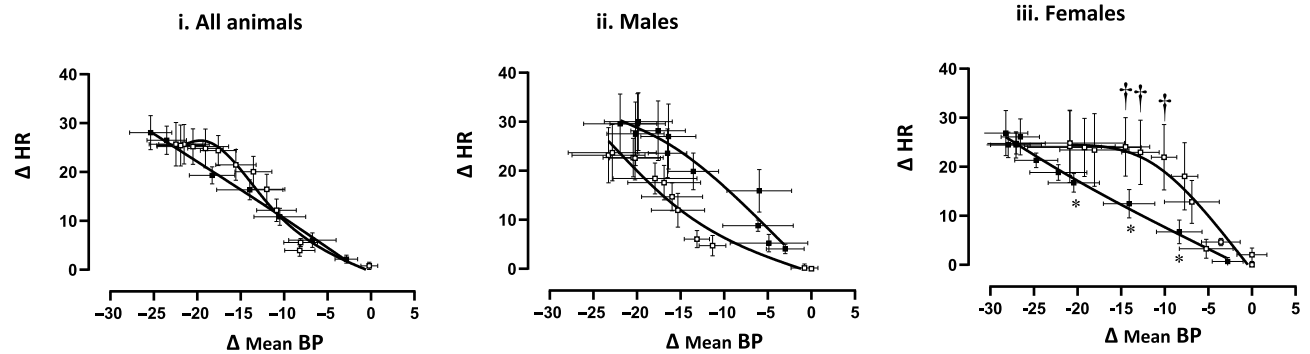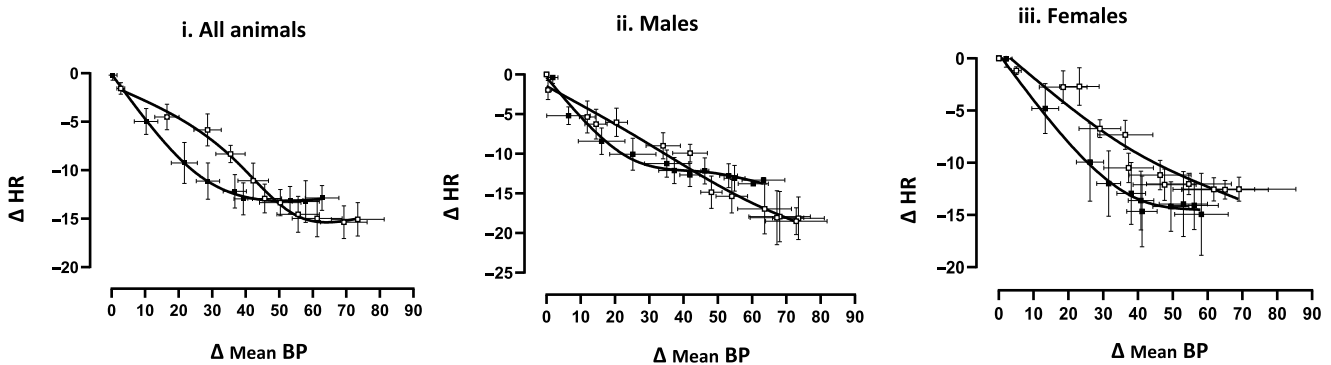

(b) Parasympathetic dominance

Fig. 4. The relative dominance of the (a) sympathetic and (b) parasympathetic components of the baroreflex curves measured as the relationship between the mean changes (±SEM on *x* and *y* axes) in mean arterial BP (mmHg) and HR (beats per minute) in response to a hypotensive challenge (SNP: 2.5 µg/kg/min) and to a hypertensive challenge (PE infusion: 6 µg/kg/min), respectively, in ponies at 2–3 years of age after neonatal treatment with either saline (open symbols, *n* = 8) and ACTH (filled symbols, *n* = 9) for (i) all animals irrespective of sex, (ii) males only (saline, *n* = 4; ACTH, *n* = 4) and (iii) females only (saline, *n* = 4; ACTH, *n* = 5). *Significantly different from saline-treated group of females (two-way ANOVA, *P* < 0.05 with Holm–Sidak *post hoc* test). †Significantly different from value in the saline-treated group of males in panel Aii (two-way ANOVA, *P* < 0.02 with the Holm–Sidak *post hoc* test).

baroreflex sensitivity appeared to be due primarily to altered parasympathetic rather than sympathetic activity and were not associated with changes in the HR range, indicative of impaired central processing of baroreceptor signals.[46,60] In the current study, neonatal cortisol overexposure reduced the gain of the baroreflex curve in the female, but not the male adult horses. This effect appeared to be due predominantly to a decrease in the sympathetic component of baroreflex control, which suggests that the normal ontogenic increase in sympathetic dominance of the baroreflex seen after birth may have been adversely affected by neonatal hypercortisolaemia.[32] However, whether these developmental changes reflect altered afferent signals, their central integration and/or responsiveness of the target organs to autonomic outflow remains unknown.

The efficacy of PE and SNP in inducing cardiovascular responses reflects not only the receptor expression and downstream signalling mechanisms but also the circulating concentration and clearance of these receptor agonists. Prenatal glucocorticoid overexposure reduces adult nephron numbers and increases glomerular filtration rate of individual nephrons in female sheep,[67] which may have implications for SNP concentrations as renal excretion is the main route of SNP clearance. However, relatively little is known about whether the renal consequences of early life glucocorticoid overexposure are sex-linked in adulthood.[47] Similarly, PE clearance may be affected by neonatal glucocorticoid overexposure as monoamine oxidase activity responsible for PE clearance is sensitive to both early life programming and the adult glucocorticoid concentration.[68,69] Previous studies in the current cohort of young adult horses have shown that the HPA response to insulin-induced hypoglycaemia is increased after neonatal cortisol overexposure.[27,28] Although this heightened response was not sex-linked,[27,30] an elevated adult cortisol concentration, particularly in response to SNP-induced hypotension, might also be a contributory factor in the altered cardiovascular function seen in the young adult ponies overexposed to cortisol neonatally. Consequently, changes in PE and SNP clearance and, hence, concentrations may also have a role in the altered cardiovascular responses seen between sexes and after neonatal cortisol overexposure in the current study.

The mechanisms underlying the sexual dimorphism of adult cardiovascular function seen in young adult horses both under basal conditions and in response to neonatal cortisol overexposure, also remain unclear. Relatively few studies have examined the long-term cardiovascular effects of early life overexposure to glucocorticoids in both sexes in any species and those that have find either little difference between the sexes or more adverse effects in males than females in older animals.[23,46,70,71] Cardiovascular function is known to be affected by puberty and increased secretion of the different gonadal steroids.[72–74] Consequently, the sexual dimorphism in the cardiovascular outcomes of neonatal cortisol overexposure in young 2- to 3-year-old ponies may, in part, reflect the earlier onset of puberty in fillies, since full sexual maturity is reached by 2 years in fillies but occurs up to a year later in colts.[75] Differential effects of neonatal glucocorticoid treatment on

cardiovascular function are also seen in peri-pubertal boys and girls born pre-term.[50] Collectively, these findings suggest that, in long-lived species, sex-linked differences in cardiovascular function may be more obvious after puberty due to the cardio-protective effects of oestrogens.[76,77] Certainly, the elevated basal diastolic pressure and depressed gain of the cardiac baroreflex in control colts relative to control fillies may reflect a greater propensity for basal arterial BP to be easily stimulated in males relative to females and is consistent with the greater susceptibility of men than women to hypertension and cardiovascular dysfunction in mid-life.[78,79]

In summary, the current study is the first to report the long-term cardiovascular effects of neonatal glucocorticoid overexposure in horses. It shows that there is a window of susceptibility for glucocorticoid programming of cardiovascular function in the immediate neonatal period in horses that is sex-specific. This is consistent with the endocrine and metabolic programming observed in previous studies of these animals.[28,29] The cardiovascular dysfunction measured in the young adult horses treated with ACTH after birth occurred without alterations in basal BP, which suggest that the abnormalities are not the consequence of hypertension but are more likely to be a primary defect in development of the heart and/or the blood vessels programmed by the neonatal overexposure to cortisol. Further studies are needed to determine whether these cardiovascular changes persist and develop into overt hypertension and cardiovascular disease with increasing age. However, the present findings *per se* have important implications for the health and athletic performance of the population of young adult horses involved in racing and other sports, particularly if they have been clinically or naturally overexposed to glucocorticoids in the neonatal period.

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