

## ORIGINAL ARTICLE

## Human fetal and maternal corticotrophin releasing hormone responses to acute stress

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**Objectives:** To study the effect of acute stress, caused by intrauterine needling at the intrahepatic vein (IHV), on fetal plasma concentrations of corticotrophin releasing hormone (CRH), and to compare paired fetal and maternal samples for CRH concentration to determine the extent of their joint control.

**Design:** Venous blood samples were obtained from fetuses (gestational age 17–38 weeks) undergoing fetal blood sampling (n = 29) or intrauterine transfusion (n = 17) through either the IHV or the placental cord insertion (PCI).

**Setting:** The Centre for Fetal Care, Queen Charlotte's and Chelsea Hospital, London, UK.

**Patients:** Pregnant women undergoing clinically indicated fetal blood sampling or intrauterine blood/platelet transfusion.

**Results:** Fetal plasma cortisol increased with intrahepatic vein transfusion (mean (SD) cortisol response  $\Delta 64.7$  (54.5) nmol/l;  $p < 0.0001$ , n = 11), and fetal corticotrophin concentrations were higher after IHV (n = 7) than PCI needling (n = 6). Neither fetal nor maternal plasma CRH increased after IHV transfusion. Fetal CRH levels did not rise with gestation, whereas maternal CRH levels did ( $r = 0.58$ ; n = 36;  $p < 0.0001$ ). There was a modest correlation between paired maternal and fetal values ( $r = 0.36$ ; n = 36;  $p = 0.03$ ).

**Conclusions:** Acute fetal stress, caused by IHV needling of the fetal abdomen, resulted in hypothalamic-pituitary-adrenal axis activation, as shown by a rise in fetal cortisol and corticotrophin. However, it did not result in measurable CRH release into fetal plasma. This suggests that fetal plasma CRH is not derived from the hypophyseal-portal circulation, but from another source, presumably the placenta.

Although corticotrophin releasing hormone (CRH) is normally very low in adult plasma, maternal concentrations increase substantially during pregnancy, with the largest rise in the two weeks before delivery.<sup>1–3</sup> High maternal levels have been associated with preterm labour.<sup>4</sup> Although CRH is present in fetal plasma,<sup>2</sup> few studies have measured fetal CRH,<sup>2 5 6</sup> and none have examined levels in response to stress.

We report CRH concentrations in human fetal venous samples obtained by needling at the placental cord insertion (PCI) or the intrahepatic vein (IHV), either before and after transfusion or from a single fetal blood sampling procedure. The PCI is not innervated, and we have shown in previous studies that needling at this site is not stressful for the fetus, whereas needling through the IHV, which involves piercing the fetal abdomen, is stressful.<sup>7–9</sup> Such procedures using the IHV cause a rise in fetal venous concentrations of the stress hormones cortisol,  $\beta$  endorphin, and noradrenaline (norepinephrine), and also Doppler changes suggestive of blood flow redistribution to the brain.<sup>7–10</sup>

Our hypothesis was that, unlike other stress hormones, fetal peripheral blood levels of CRH would not rise after needling at the IHV, as stress related release of CRH should be confined to the hypophyseal-portal circulation, and, as in the adult, not reach the plasma in any measurable quantity.<sup>3</sup> This would be in contrast with the suggestion of Lockwood *et al*<sup>6</sup> that some fetal plasma CRH may derive from the fetal hypothalamus.

Maternal samples were obtained at the same time as the fetal samples, to determine the relation between maternal and fetal CRH, because of the possibility that they are jointly influenced by placental as opposed to hypothalamic CRH, or that there is transplacental passage from mother to fetus, as we have previously shown for cortisol.<sup>11</sup> Finally, we con-

firmed fetal hypothalamic-pituitary-adrenal axis responsivity to acute stress by measuring cortisol and corticotrophin concentrations.<sup>7 9</sup> Although corticotrophin has been measured in fetal blood,<sup>5 6</sup> the fetal corticotrophin response to a stressful procedure has not.

## MATERIALS AND METHODS

## Patients

Women undergoing clinically indicated fetal blood sampling or intrauterine blood/platelet transfusion at the Centre for Fetal Care, Queen Charlotte's and Chelsea Hospital, London, UK were recruited. Ethical approval for the study was granted by the Hammersmith Hospitals ethics committee. Cortisol and  $\beta$  endorphin results from some of these fetuses have been reported previously.<sup>9</sup>

Inclusion criteria were singleton fetuses appropriately grown for gestational age and structurally normal on ultrasound. Exclusion criteria were similar to those used in our previous studies.<sup>7–10</sup> The indications for fetal blood sampling and for intrauterine transfusion were also similar to those used previously.<sup>7–10</sup>

The site of ultrasound guided fetal blood sampling or transfusion was chosen by the operator on the basis of technical factors and ease of approach. Neither fetal neuromuscular blockade nor analgesia was used. The mothers did not receive sedation. Intravascular transfusion was performed as previously described.<sup>7–10</sup> Thirty two fetuses underwent procedures at the IHV, 11 of these receiving a blood transfusion. Fetal blood was obtained from 14 PCI procedures, six receiving blood transfusions. Mean (SD) duration of trans-

**Abbreviations:** CRH, corticotrophin releasing hormone; IHV, intrahepatic vein; PCI, placental cord insertion

**Table 1** Characteristics of blood samples

	Before IHV transfusion	Before PCI transfusion	Needling
Number	16	6	66
Gestational age (weeks)	29.1 (5.10)	30.5 (4.59)	28.4 (4.88)
Packed cell volume	31.6 (7.11)	29.8 (5.46)	34.0 (7.20)
Cortisol (nmol/l)	60.7 (41.8 to 88.2)	50.6 (9.63 to 266)	57 (50.0 to 65.4)
CRH (pg/ml)	219 (191 to 250)	210 (163 to 271)	210 (47.6)
Maternal cortisol (nmol/l)	719 (613 to 843)	769 (598 to 989)	736 (240)
Maternal CRH (pg/ml)	368 (293 to 463)	377 (313 to 454)	334 (305 to 366)
Time to access (min)	3.02 (1.84 to 4.96)	2.74 (0.91 to 8.23)	3.40 (2.63 to 4.41)
Duration (min)	17.9 (10.1)	11.9 (5.49)	

Values are mean (SD) or geometric mean (anti-ln 95%CI).

IHV, Intrahepatic vein; PCI, placental cord insertion; CRH, corticotrophin releasing hormone.

fusion was 16.5 (9.8) minutes, and the (geometric) mean (anti-ln 95% confidence interval) duration of needling to access the fetal circulation was 4.26 (3.26 to 5.58) minutes.

### Blood samples

After collection of clinical samples, up to 1–2 ml venous fetal blood was placed in a chilled heparinised tube. If there was sufficient, an additional 0.5–1 ml was placed into a chilled EDTA tube for corticotrophin determination. Maternal blood (7 ml) was collected by venepuncture into a heparinised Vacutainer (Becton Dickinson, Meylan, France) immediately before transabdominal needle insertion and again 10–20 minutes after the procedure.

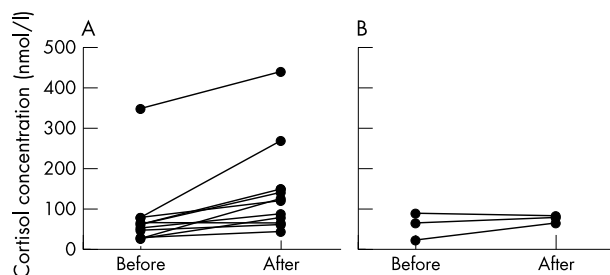
Trasylol (50 µl/ml (500 KIU); Bayer AG) was added to all blood samples immediately after collection. Bloods were prepared as described previously.<sup>9</sup>

### Assays

Plasma CRH was assayed using a direct plasma radioimmunoassay (Peninsula, San Carlos, California, USA). The lower limit of sensitivity was 10 pg/ml, and the assay coefficient of variation was 10.5%. Cortisol was assayed using a standard solid phase radioimmunoassay (DPC, Los Angeles, California, USA); the lower limit of sensitivity was 10 nmol/l, and the assay coefficient of variation was 5.3%. Maternal and fetal plasma sample pairs were analysed in the same assay run. In general, cortisol was measured in parallel, but in a few cases, there was insufficient sample volume to measure cortisol as well as CRH. Limited sample volume also meant it was only possible to determine corticotrophin in a small number of blood samples taken before transfusions. EDTA plasma corticotrophin was assayed using a standard solid phase two site immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, California, USA). The lower limit of sensitivity was 1.0 pg/ml, and the assay coefficient of variation was 8.6%.

### Statistical analysis

Normally distributed data were analysed by standard parametric statistics using SPSS 10.0 for Windows (Chicago,



**Figure 1** Fetal cortisol levels before and after transfusion at the intrahepatic vein (A) (paired *t* test  $p < 0.001$ ;  $n = 11$ ) and at the placental cord insertion (B) (paired *t* test  $p = 0.3$ ;  $n = 3$ ).

Illinois, USA). Non-parametric initial values (fetal cortisol and maternal CRH levels, time to access) and maternal and fetal transfusion data were first normalised by natural log transformation. Geometric means and anti-ln 95% confidence intervals (95%CI) are displayed. Responses to transfusions were analysed using  $\Delta$  values (hormone concentration after transfusion stress – hormone concentration before transfusion stress).

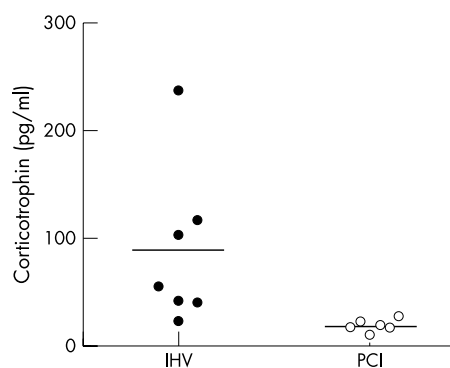
## RESULTS

### Fetal samples

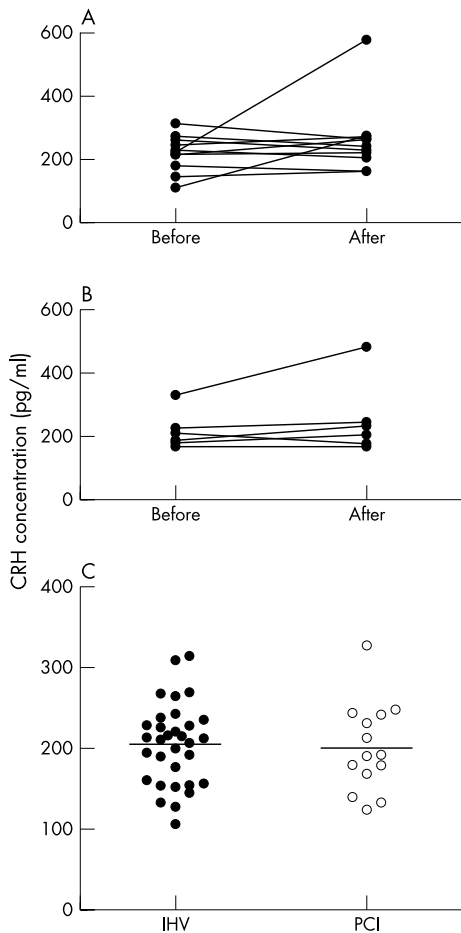
Table 1 gives details of the pre-transfusion and needling samples. Fetal plasma cortisol concentrations increased with transfusion at the IHV (fig 1A); mean (SD)  $\Delta 64.7$  (54.5) nmol/l ( $n = 11$ ,  $p < 0.0001$ ). The few transfusions at the PCI with sufficient sample ( $n = 3$ ) showed no significant change (fig 1B). Corticotrophin levels were significantly higher in the needling only—that is, non-transfusion—samples from the IHV than from the PCI (88.1 (73.6) pg/ml ( $n = 7$ ) *v* 18.7 (5.7) pg/ml ( $n = 6$ );  $p = 0.04$ ) (fig 2). There was insufficient plasma volume available to assay corticotrophin in the transfusion group samples.

Figures 3A and 3B show that there was no change in fetal CRH level with transfusion at either the IHV or PCI. Similarly, fetal CRH concentrations were similar after needling at the two sites (fig 3C).

There was no significant difference in  $\Delta$  fetal CRH levels in procedures that involved transplacental insertion of the needle (median  $\Delta$  15 pg/ml; range (–40 to 163);  $n = 11$ ) and those that did not (median  $\Delta$  –4 pg/ml; (–51 to 362);  $n = 6$ ). Similarly, there was no significant difference in initial fetal CRH levels in procedures that involved transplacental insertion of the needle (mean (SD) 185 (49.4) pg/ml;  $n = 20$ ) and those that did not (216 (48.0) pg/ml;  $n = 26$ ).



**Figure 2** Fetal corticotrophin levels after blood samplings from the intrahepatic vein (IHV) and placental cord insertion (PCI) sites (*t* test  $p = 0.04$ ; IHV,  $n = 7$ ; PCI,  $n = 6$ ).

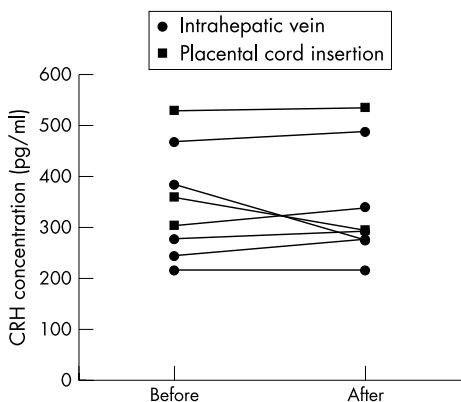


**Figure 3** Fetal corticotrophin releasing hormone (CRH) concentration (A) before and after transfusion at the intrahepatic vein (IHV) site (paired *t* test *p* = 0.2; *n* = 11), (B) before and after transfusion at the placental cord insertion (PCI) site (paired *t* test *p* = 0.3; *n* = 6), and (C) from IHV and PCI blood samplings (unpaired *t* test *p* = 0.9; IHV, *n* = 32; PCI, *n* = 14).

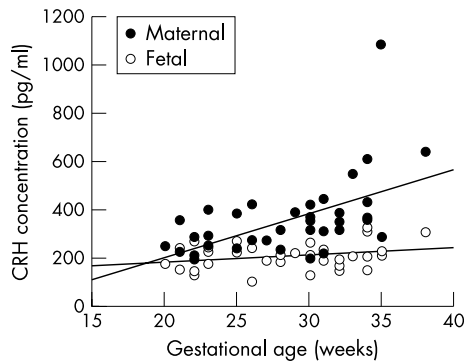
**MATERNAL AND FETAL CRH**

Maternal CRH levels did not change with transfusion at either site (fig 4).

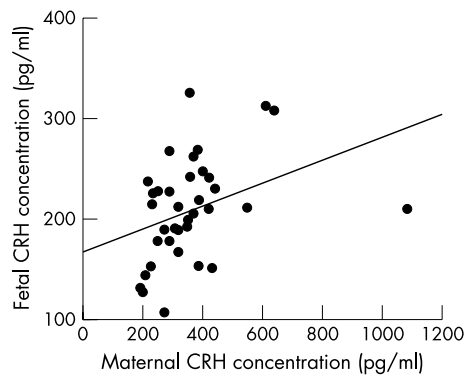
Figure 5 shows the relation between paired fetal and maternal CRH levels and gestational age between 20 and 38 weeks. Whereas maternal levels increased substantially with advancing gestation, fetal levels did not (maternal *r* = 0.58, *n* = 36, *p* < 0.0001; *y* = 98.4+1.04 anti-ln *x*; anti-



**Figure 4** Maternal corticotrophin releasing hormone (CRH) levels before and after transfusion (paired *t* test *p* = 0.8; *n* = 8).



**Figure 5** Relation between paired maternal and fetal corticotrophin releasing hormone (CRH) levels and gestational age (*n* = 36). Maternal CRH, Pearson *r* = 0.58, *p* < 0.0001, *y* = 98.4+1.04 anti-ln *x*, anti-ln 95%CI for slope (1.02 to 1.07); fetal CRH, Pearson *r* = 0.27, ns.



**Figure 6** Paired maternal and fetal corticotrophin releasing hormone (CRH) levels. Pearson *r* = 0.36, *p* = 0.03, *n* = 36, *y* = 170+0.11*x*, (0.01 to 0.22).

In 95%CI for slope (1.02 to 1.07); fetal *r* = 0.27). Figure 6 shows a modest correlation between paired fetal and maternal CRH levels (*r* = 0.36, *n* = 36, *p* = 0.03; *y* = 170+0.11*x*; (0.01 to 0.22)). This correlation remained significant even on removal of the outlier.

There was no significant relation between initial maternal or fetal CRH and cortisol levels, except for a correlation between fetal CRH and fetal cortisol levels (*r* = 0.32, *n* = 38, *p* = 0.048; table 2).

**DISCUSSION**

This study confirmed our hypothesis, in that needling the IHV was not associated with an increase in fetal plasma CRH levels, either after transfusions, which we confirmed are associated with an increase in cortisol (fig 1), or after fetal blood sampling only procedures, which we showed were associated with an increase in corticotrophin (fig 2). This shows that the fetal hypothalamus-pituitary-adrenal axis,

**Table 2** Correlations between maternal and fetal corticotrophin releasing hormone (CRH) and cortisol levels (after needling or before transfusion)

	Maternal CRH	Fetal CRH
Maternal cortisol	<i>r</i> = -0.002; <i>n</i> = 33; ns	<i>r</i> = 0.04; <i>n</i> = 31; ns
Fetal cortisol	<i>r</i> = 0.11; <i>n</i> = 32; ns	<i>r</i> = 0.32; <i>n</i> = 38; <i>p</i> = 0.048

although functional from mid-gestation,<sup>9</sup> does not involve any measurable release of CRH into fetal plasma. This is similar to the situation in adults.

The finding that transfusion at the IHV leads to a rise in fetal cortisol supports our previous findings,<sup>7-9</sup> and the lack of effect on fetal CRH levels indicates that placental CRH does not mediate the acute response to the stress of percutaneous needling in the human fetus.

The corticotrophin response to stress has not been previously studied in the human fetus. The increase in levels at the IHV but not the PCI, after needling, is consistent with a more rapid release than that of cortisol. This is compatible with release from the pituitary rather than the adrenal, and is also similar to findings in adults and animal models.<sup>12</sup> In a previous study we have shown that noradrenaline, which is also released more quickly than cortisol, was increased in initial values after IHV fetal blood sampling compared with PCI.<sup>8</sup>

Our results suggest that fetal plasma CRH is derived from another source, presumably the placenta. It is well established that the placenta produces large amounts of CRH<sup>4 13-16</sup> and that both maternal and fetal aspects of the syncytiotrophoblast contain binding proteins for CRH, with significantly more on the maternal side.<sup>17-19</sup> This study shows that, whereas maternal plasma CRH levels increased substantially with gestation, fetal levels did not (fig 5); indeed, although there was some joint control of fetal and maternal CRH levels, it was not substantial ( $r = 0.36$ ). The results suggest that the placenta secretes CRH preferentially into the maternal rather than the fetal circulation as gestation increases.<sup>20</sup> Our results do not preclude a fetal plasma CRH rise in the last two weeks of gestation, or infection or hypoxic stress mediating a fetal CRH rise immediately before delivery, although Lockwood *et al*<sup>6</sup> found no increase up to 40 weeks gestation.

It is unlikely that there is any substantial direct maternal to fetal transfer across the placenta, both because of the nature of CRH (a 41 amino acid peptide) and the divergent patterns of maternal and fetal cortisol with increasing gestation. Our results in this regard are similar to those of Lockwood *et al*.<sup>6</sup> Our correlation between maternal and fetal levels of 0.36 is also in the same range as that found in two previous studies.<sup>5 6</sup>

It is of interest that maternal CRH did not change with transfusion. There is sometimes concern that transplacental needling procedures may induce preterm labour through increased release of placental CRH. We found no evidence of this, although increased CRH release over a longer time period than measured immediately after transfusion remains a possibility.

The lack of correlation between maternal CRH and cortisol supports the findings of previous studies.<sup>1 2 21</sup> However, Lockwood *et al*<sup>6</sup> found a correlation of 0.41 between maternal CRH and fetal cortisol, which we did not observe here. Our finding of a significant correlation of 0.32 between fetal CRH and fetal cortisol is of interest, and may suggest joint control in the fetal compartment, either possibly driving the other. However, as Lockwood *et al*<sup>6</sup> failed to find such a correlation, our finding must be interpreted with caution. Also, maternal peptides may elicit fetal effects, through release of placental hormones into the fetal compartment.<sup>22</sup>

Lockwood *et al*<sup>6</sup> have suggested that the dissociation between maternal and fetal plasma CRH levels was because a proportion of the fetal CRH derived from the fetal hypothalamus. We show here that this is unlikely, and suggest, in contrast, that the plasma CRH in both circulations derives from the placenta, where they are, at least partially, under different control mechanisms. The fact that fetal plasma CRH, unlike maternal CRH, does not rise substantially with gestation, at least until 38 weeks, makes it unlikely that it is involved in the normal triggering of parturition. One suggestion is that it stimulates adrenal

dehydroepiandrosterone sulphate, which in turn acts as a precursor for oestradiol.<sup>23</sup> At present, however, the role of fetal plasma CRH remains obscure.

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