Development of insulin and proinsulin secretion in newborn pony foals

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Abstract

At birth, the endocrine pancreas must assume a glucoregulatory role if the neonate is to survive the transition from parenteral to enteral nutrition. In species like the horse, neonatal hypoglycaemia is common, which suggests that the glucoregulatory mechanisms are not always fully competent at birth. Hence, this study examined pancreatic β cell function in newborn foals during nutritional adaptation over the first 10 days *post partum*. Over a 48 h period at three time intervals after birth (days 1–2, 5–6 and 9–10 *post partum*), the β cell responses to suckling and to intravenous administration of glucose, arginine and saline were measured in seven normal pony foals. Basal plasma concentrations of proinsulin, but not insulin or glucose, increased significantly between days 1 and 10. Suckling caused a gradual increase in plasma glucose, which was accompanied by a significant increase in plasma insulin concentrations 15 min after the onset of suckling on days 5 and 9, but not day 1. There was no significant change in plasma proinsulin concentrations in response to suckling at

any age. At all ages studied, glucose and arginine administration stimulated an increase in the plasma concentrations of insulin and proinsulin; these β cell responses did not change significantly with postnatal age. The insulin responses to glucose were significantly greater than those of arginine at each time period. Glucose clearance was significantly slower on day 1 than subsequently. Proinsulin and glucose, but not insulin, concentrations decreased significantly after saline administration at all three ages. At each time period, there was a significant positive relationship between the plasma insulin and proinsulin concentrations, the slope of which was significantly shallower on days 1–2 than subsequently. These results show that equine β cells are responsive to glucose and arginine and release both insulin and proinsulin during the immediate postnatal period. They also suggest that newborn foals may be insulin resistant on the first day after birth.

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Introduction

At birth, the continuous placental supply of nutrients ceases and is replaced by an intermittent supply of substrates via the gut (Trahair & Sangild 1997, Fowden *et al.* 1998). During this switch from parenteral to enteral nutrition, maintenance of normoglycaemia depends, in part, on the glucoregulatory endocrine glands (Fowden *et al.* 2001). Prenatally, fetal glucose concentrations are controlled primarily by maternal glucose availability and, hence, endocrine glands such as the pancreas are less directly involved in glucose homeostasis before than after birth. However, the endocrine pancreas must be able to assume a glucoregulatory role shortly after birth, if the neonate is to survive the transition to enteral feeding (Trahair & Sangild 1997, Fowden *et al.* 2001). In some species, such as the horse, episodes of hypoglycaemia are

common during the neonatal period (Spurlock & Furr 1990, Madigan 1991), which suggests that the glucoregulatory mechanisms are not always fully competent at birth.

The endocrine pancreas is functional and responsive before birth in all species studied to date, including the horse (Fowden *et al.* 1980, Fowden 1985). It secretes insulin in response to glucose and amino acids and has a central role in regulating fetal nutrient utilization and growth (Fowden 1995). Insulin is derived from the larger precursor molecule, proinsulin, by cleavage within the secretory granules of the fetal β cells (Orsi *et al.* 1971) and is released in response to membrane depolarization induced either directly or indirectly via generation of ATP (Fowden & Hill 2001). Compared with adult plasma, fetal plasma has been found to contain little proinsulin during late gestation, although both intact and split proinsulin are

present in cord plasma from human infants at birth (Godfrey *et al.* 1996). In sheep, the pancreatic β cells become progressively more responsive towards term and are very sensitive to changes in glycaemia in the immediate neonatal period (Phillips *et al.* 1981, Aldoretta *et al.* 1998). Similarly, in horses, there are increases in pancreatic β cell sensitivity to glucose during late gestation and, again, between birth and 7 days of postnatal age (Fowden *et al.* 1980, 1982). However, little is known about the changes in neonatal β cell function and proinsulin secretion as the glucoregulatory demands for insulin increase during the establishment of enteral feeding. The aim of this study was, therefore, to examine the insulin and proinsulin responses of newborn foals to endogenous and exogenous stimuli during the critical period of glucoregulatory adaptation in the first 10 days after birth.

Material and Methods

Animals

Seven pony foals were used in the study. They were born spontaneously at full term (gestational age range 326–345 days) and classified as normal, mature foals using clinical criteria (Rossdale *et al.* 1984). Their mean ($\pm$ S.E.) birth weight was 20.3 ± 1.1 kg, and all gained 1–2 kg per day throughout the 10-day experimental period. The mares delivered unassisted and had normal placentae. The foals were housed with their mothers in individual boxes throughout the experimental period. They suckled *ad libitum* except during the tests of pancreatic β cell function, when they were muzzled for periods of up to 90 min. All procedures were carried out under the Animals (Scientific Procedures) Act 1986.

Experimental procedures

Between 6 and 8 h after birth, a long-term polyurethane catheter (Milacath, Mila International Inc., Kentucky, USA) was inserted into a jugular vein of each foal, with the animal under mepivacaine hydrochloride local anaesthesia (Intra-Epicaine, Arnolds Veterinary Products, Shrewsbury, UK). The catheters were placed at least 1 h before any tests began on day 1 and left *in situ* for the 10 days of the protocol. The pancreatic β cell responses to the endogenous stimulus of suckling and to the exogenous stimulus of intravenous administration of glucose or arginine were measured over 48 h at three time periods after birth (period 1: days 1 and 2; period 2: days 5 and 6; period 3: days 9 and 10 *post partum*).

Endogenous stimulation On days 1, 5 and 9, the foals were muzzled for 30 min. At the end of that period, a blood sample (5–6 ml) was taken (0 min) and the muzzle

was removed to allow the foal to suckle from its mother. Further blood samples were taken at 5 and 15 min after the muzzle had been removed. All foals suckled immediately the muzzle was removed and for at least 2 min.

Exogenous stimulation Foals were allowed to suckle, and were then muzzled throughout the experimental procedure. After 30 min, either glucose (0.5 g/kg, 40% glucose, Arnolds Veterinary Products; days 1, 5 and 9) or arginine (100 mg/kg, L-arginine hydrochloride, Sigma-Aldrich Co. Ltd, in 20 ml 0.9% w/v NaCl; days 2, 6 and 10) was given intravenously to the foal. Saline (20 ml 0.9% w/v NaCl) was given as a control on days 2, 6 and 10. Blood samples (5–6 ml) were taken immediately before (0 min) and at 5, 15, 30 and 60 min after administration of the solutions. The muzzle was then removed and the foals were allowed to suckle *ad libitum*.

All blood samples were collected into both heparinized tubes (2.5 ml; insulin, proinsulin) and EDTA-containing tubes (2.5 ml; cortisol, glucose, α -aminonitrogen). In addition, 1 ml aliquots of samples collected at 0 min (all experiments) and 5 min (suckling experiment) were added to heparinized tubes containing EGTA and glutathione for the determination of plasma catecholamine concentrations (Silver *et al.* 1984). All the blood samples were centrifuged at 4 °C immediately after collection and the plasma was stored at –20 °C (insulin, proinsulin, cortisol, glucose, α -aminonitrogen) or –70 °C (catecholamines) until required for analysis.

Biochemical analyses

Metabolite concentrations Plasma glucose and α -aminonitrogen concentrations were determined colorimetrically as described previously (Prenton & London 1967, Fowden *et al.* 1982).

Hormone concentrations Plasma insulin and proinsulin concentrations were assayed on a 1235 AutoDELFIa automatic immunoassay system using a two-step time-resolved fluorimetric assay. The principles of DELFIA (Dissociation Enhanced Lanthanide Fluoroimmunoassay) have been described previously (Hemmilä *et al.* 1984). All reagents, standards and consumables were those recommended and supplied by the manufacturer (Wallac Oy, Turku, Finland). Insulin concentrations were assayed using AutoDELFIa insulin kits (Kit no. B080–101, Wallac Oy) as described by Andersen and co-workers (1993). Interassay coefficients of variation were 3.1% at 29.0 pmol/l, 2.1% at 79.4 pmol/l, 1.9% at 277 pmol/l and 2.0% at 705 pmol/l. The limit of detection of the insulin assay was 1.3 pmol/l and the assay was linear to approximately 1100 pmol/l. Cross-reactivities were: intact proinsulin <0.5% at 2736 pmol/l, 32–33 split proinsulin 1% at 2800 pmol/l and C-peptide <0.1% at 5280 pmol/l. Total proinsulin concentrations were determined using

Table 1 Mean ($\pm$ S.E.) basal concentrations of metabolites and hormones in seven newborn pony foals with respect to postnatal age

	Day 1	Day 2	Day 5	Day 6	Day 9	Day 10
Glucose (mmol/l)	8.12 $\pm$ 0.45	10.53 $\pm$ 0.74	8.86 $\pm$ 0.51	9.97 $\pm$ 0.68	8.74 $\pm$ 0.63	10.05 $\pm$ 0.82
α -Aminonitrogen (mmol/l)	2.23 ^a $\pm$ 0.13	3.78 ^b $\pm$ 0.33	3.56 ^b $\pm$ 0.22	3.71 ^b $\pm$ 0.34	3.11 ^b $\pm$ 0.224	3.45 ^b $\pm$ 0.36
Adrenaline (pg/ml)	29.7 $\pm$ 9.9	39.3 $\pm$ 12.3	73.1 $\pm$ 19.3	59.9 $\pm$ 16.3	50.3 $\pm$ 9.7	37.9 $\pm$ 10.2
Noradrenaline (pg/ml)	223.8 ^a $\pm$ 21.9	209.0 ^{ab} $\pm$ 18.1	180.2 ^{ab} $\pm$ 39.7	182.0 ^{ab} $\pm$ 21.0	162.4 ^{ab} $\pm$ 22.0	148.6 ^b $\pm$ 18.5
Insulin (pmol/l)	91.9 $\pm$ 29.2	147.1 $\pm$ 30.5	139.1 $\pm$ 38.4	160.3 $\pm$ 31.7	111.5 $\pm$ 31.43	156.3 $\pm$ 39.5
Proinsulin (pmol/l)	7.0 ^a $\pm$ 1.1	11.5 ^{ab} $\pm$ 2.7	14.9 ^{bc} $\pm$ 3.3	16.6 ^c $\pm$ 3.3	16.2 ^c $\pm$ 3.1	17.2 ^c $\pm$ 3.2

Values within rows with different superscript letters are significantly different from each other (one-way ANOVA with repeated measures, $P < 0.05$).

an in-house AutoDELFI method developed at the Department of Clinical Biochemistry, Addenbrookes NHS Trust, Cambridge, UK. The solid-phase capture antibody (3B1), bound to a microtitre plate, was specific for the proinsulin B-chain (Sobey *et al.* 1989). The detection antibody, specific for the proinsulin C-chain (CPT-3F11) was kindly donated by DakoCytomation Ltd, Angel Drove, Ely, UK. This antibody was subsequently labelled with Europium using the DELFIA Europium labelling kit (no. 1244-302, Wallac Oy). The assay was calibrated with chromatography-purified 32-33 split proinsulin standard, kindly donated by Lilly Research Laboratories (Indianapolis, Indiana, USA). The total proinsulin assay showed 100% cross-reactivity with human intact proinsulin and 32-33 split proinsulin and $< 1\%$ cross-reactivity with human insulin at 2500 pmol/l. This assay, therefore, measured the total concentration of both intact and split proinsulin. Coefficients of variation were 7.6% at 6.3 pmol/l, 6.5% at 14.0 pmol/l and 3.9% at 79.9 pmol/l. The limit of detection of the proinsulin assay was less than 1.25 pmol/l. The assay range, without dilution, was approximately 400 pmol/l. Samples with analyte concentrations greater than the dynamic range of the assays were reanalysed after dilution in a protein-based buffer (DELFI II Wallac Oy). The insulin and proinsulin concentrations in equine plasma stripped of hormones by charcoal treatment were below the limit of detection for both assays. Dilution of equine plasma with high insulin and proinsulin concentrations using stripped equine plasma produced curves parallel to the standard curves in each assay.

Plasma adrenaline and noradrenaline concentrations were measured by HPLC as described previously (Silver *et al.* 1987). Briefly, plasma samples were prepared by absorption of 300–500 μ l onto acid-washed alumina and 20 μ l samples of the 100 μ l perchloric acid eluate were injected onto the column. Before absorption, dihydroxybenzylamine was added to each plasma sample as internal standard. Standard solutions of adrenaline and noradrenaline were also treated in the same way. The limits of sensitivity were 0.07 ng/ml for adrenaline and 0.05 ng/ml for noradrenaline. The intra- and inter-assay coefficients of variation were 5.8% and 7.3% for adrenaline and 5.0% and 6.2% for noradrenaline respectively.

Statistical analyses

Means ($\pm$ S.E.) are given throughout. Statistical analyses were performed using SigmaStat Statistical Software version 2.0. Statistical significance was assessed using parametric tests, including one- and two-way ANOVA with repeated measures and Student's *t*-test as appropriate. For each experiment, the area under the curve (AUC) for the insulin, proinsulin, glucose and α -aminonitrogen responses were calculated as the integrated plasma concentrations after administration of glucose or arginine, from 5 to 60 min above the baseline concentration at 0 min for all positive values. The relationships between the plasma concentrations of insulin and proinsulin were assessed using linear regression analysis. For all statistical tests, significance was accepted at $P < 0.05$.

Results

Basal metabolite and hormone concentrations

The samples collected at 0 min after the foals had been muzzled for 30 min on days 1, 2, 5, 6, 9 and 10 were used to assess changes in the basal metabolite and hormone concentrations over the first 10 days of postnatal life. There were no significant changes in the glucose concentrations with age after birth (Table 1). In contrast, plasma α -aminonitrogen concentrations increased significantly ($P < 0.002$) between day 1 and 2 and then remained stable throughout the remaining 10-day period (Table 1). Plasma adrenaline concentrations were near or below the limit of detection of the assay on all days studied, whereas plasma noradrenaline concentrations were greatest on day 1 and decreased progressively ($P < 0.014$) over the 10 days of the study (Table 1). Basal plasma insulin concentrations showed no significant change with postnatal age, whereas total proinsulin concentrations increased significantly between days 1 and 5 and then remained stable (Table 1). There was no relationship between birth weight and the basal concentration of either insulin ($r = 0.376$, $n = 7$, $P > 0.05$) or total proinsulin ($r = 0.301$, $n = 7$, $P > 0.05$) on day 1 or subsequently ($P > 0.05$, all cases).

Figure 1 Mean ($\pm$ S.E.) increments in the plasma concentration of (A) glucose, (B) insulin and (C) total proinsulin after the onset of suckling at 0 min in seven pony foals on day 1 (●), day 5 (◐) and day 9 (○) of postnatal life. *Significant increment from 0 min value (one-way ANOVA with repeated measures, $P < 0.05$).

Pancreatic β cell responses to endogenous stimuli

Suckling caused a gradual increase in the plasma glucose concentration on days 5 and 9, but not on day 1 (Fig. 1A). Mean plasma glucose concentrations were significantly greater than the basal values 15 min after the onset of suckling on days 5 and 9, but remained unchanged from baseline throughout the sampling period on day 1 (Fig. 1A). Plasma insulin concentrations increased in parallel with the glucose concentrations and were significantly greater than basal values 15 min after the onset of suckling on days 5 and 9, but not day 1 (Fig. 1B). The increments in plasma glucose and insulin evoked by suckling were similar on days 5 and 9 (Fig. 1A, B). There was no significant change in plasma proinsulin

Figure 2 Mean ($\pm$ S.E.) increments in the plasma concentration (A) glucose, (B) insulin and (C) total proinsulin in response to intravenous glucose (0.5 g/kg in a 40% solution) given at time 0 in seven normal pony foals on day 1 (●), day 5 (◐) and day 9 (○) of postnatal life. *Significant increment from 0 min value (one-way ANOVA with repeated measures, $P < 0.05$). Arrow indicates glucose administration.

concentrations during the 15 min period after the onset of suckling in any of the three age groups (Fig. 1C). Plasma concentrations of adrenaline and noradrenaline were not significantly different from the basal values 5 min after the onset of suckling (data not shown).

Pancreatic β cell responses to exogenous stimuli

Glucose administration Administration of glucose caused a significant increment in the plasma glucose concentration, which was maximal at 5 min (Fig. 2A) and of similar magnitude in the three age groups (Table 2). Glucose concentrations decreased rapidly thereafter and were not significantly different from baseline values by 30 min after administration on days 5 and 9. On day 1, glucose concentrations remained increased at 30 min, but had returned to basal values by 60 min after administration (Fig. 2A). The glucose AUC was, therefore, significantly greater on day 1 than on days 5 and 9 (Table 2). Glucose administration also evoked a rapid release of insulin at all three ages, with peak insulin concentrations at 5–15 min

Table 2 Mean ($\pm$ S.E.) values of the maximum increment (Δ) and AUC for plasma glucose, α -aminonitrogen, insulin and proinsulin in response to the exogenous administration of glucose or arginine in seven newborn pony foals with respect to postnatal age

	Age (days)	Glucose or α -aminonitrogen		Insulin		Proinsulin	
		Maximum Δ (mmol/l)	AUC (mmol/min per l)	Maximum Δ (pmol/l)	AUC (pmol/min per l)	Maximum Δ (pmol/l)	AUC (pmol/min per l)
Glucose	1	18.7 $\pm$ 2.8	376 $\pm$ 32 ^a	149 $\pm$ 26	3673 $\pm$ 800	5.9 $\pm$ 1.4	236 $\pm$ 72
	5	19.0 $\pm$ 1.6	246 $\pm$ 31 ^b	154 $\pm$ 30	2800 $\pm$ 599	9.8 $\pm$ 2.8	194 $\pm$ 59
	9	15.8 $\pm$ 0.7	189 $\pm$ 22 ^b	210 $\pm$ 52	3870 $\pm$ 861	12.4 $\pm$ 2.1	282 $\pm$ 52
Arginine	2	3.65 $\pm$ 0.49	56 $\pm$ 9	68 $\pm$ 19	772 $\pm$ 297	4.5 $\pm$ 1.6	106 $\pm$ 43
	6	4.00 $\pm$ 0.28	50 $\pm$ 6	88 $\pm$ 14	1495 $\pm$ 452	8.9 $\pm$ 2.5	240 $\pm$ 101
	10	3.86 $\pm$ 0.39	44 $\pm$ 9	113 $\pm$ 22	1778 $\pm$ 601	7.9 $\pm$ 1.8	265 $\pm$ 107

Within a treatment, values in a column with different superscript letters are significantly different from each other (one-way ANOVA with repeated measures, $P < 0.05$).

(Fig. 1B). The mean maximum increment in plasma insulin and the insulin AUC did not differ significantly with postnatal age (Table 2). The ratio of the insulin AUC to glucose AUC increased progressively with postnatal age and was significantly greater on day 9 (21.8 ± 4.6 , $n=7$) than on day 1 (9.7 ± 1.5 , $n=7$; $P < 0.033$). The glucose-induced increment in plasma insulin was accompanied by a significant increase in the plasma proinsulin concentration at all three ages (Fig. 2C). Proinsulin concentrations peaked 15 min after the administration of glucose and declined slowly thereafter (Fig. 2C). The mean maximum increment in plasma proinsulin in response to glucose tended to increase with postnatal age, but this trend was not statistically significant ($P=0.059$; Table 2). The proinsulin AUC did not differ significantly with postnatal age (Table 2). The insulin AUC and proinsulin AUC were significantly correlated on day 1, but not on days 5 and 9 (day 1: $r=0.934$, $n=7$, $P < 0.002$; day 5: $r=0.610$, $n=7$, $P > 0.05$; day 9: $r=0.421$, $n=7$, $P > 0.05$).

Arginine administration Administration of arginine increased plasma α -aminonitrogen concentrations to a similar extent in the three age groups (Fig. 3A). The mean maximum increase in plasma α -aminonitrogen and the α -aminonitrogen AUC in response to arginine were similar on days 2, 6 and 10 (Table 2). Arginine administration evoked a rapid release of insulin, with peak insulin concentrations at 5–15 min (Fig. 3B). The mean maximum increment in plasma insulin and the insulin AUC in response to arginine tended to increase with postnatal age, but these trends were not statistically significant ($P > 0.05$; Table 2). The maximum increment in plasma insulin and the insulin AUC were smaller in response to arginine than to glucose at each time period ($P < 0.02$ in all cases; Table 2). There was also a gradual increase in the plasma proinsulin concentration in response to arginine at all three ages (Fig. 3C), which was similar in magnitude and time course to the proinsulin response to exogenous glucose at each time period (Fig. 2C). The mean maximum increment in plasma proinsulin and the proinsulin AUC in

response to arginine tended to increase with postnatal age, but these trends were not statistically significant (Table 2). The proinsulin AUC was positively correlated to the

Figure 3 Mean ($\pm$ S.E.) increments in the plasma concentration of (A) α -aminonitrogen, (B) insulin and (C) total proinsulin in response to intravenous arginine (100 mg/kg) given at 0 min in seven pony foals on day 2 (●), day 6 (◐) and day 10 (○) of postnatal life. *Significant increment from 0 min value (one-way ANOVA with repeated measures, $P < 0.05$). Arrow indicates time of arginine administration.

Figure 4 Relationship between the plasma insulin and total proinsulin concentrations in seven normal pony foals at three time periods after birth [period 1: days 1–2 (+), $y=0.051x+3.479$; period 2: days 5–6 (□), $y=0.0843x+2.9222$; period 3: days 9–10 (△), $y=0.0657x+6.722$]. Linear regression analysis showed that the gradients of the trendlines were significantly different for each time period: period 1 compared with period 2, $P<0.001$; period 1 compared with period 3, $P=0.016$; period 2 compared with period 3, $P=0.001$. Trendlines: —, period 1; ---, period 2; -.-, period 3.

AUC insulin at all three ages (day 2: $r=0.766$, $n=7$, $P<0.04$; day 6: $r=0.827$, $n=7$, $P<0.02$; day 10: $r=0.771$, $n=7$, $P<0.04$). Mean glucose concentrations decreased progressively throughout the 60 min after the administration of arginine. The mean decrement in plasma glucose was significant at 30 and 60 min and was similar in magnitude at all three ages (data not shown).

Administration of saline on days 2, 6 and 10 had no significant effect on the plasma α -aminonitrogen or insulin concentrations: mean values did not change significantly from basal values during the 60 min sampling period (data not shown). In contrast, plasma glucose concentrations were significantly lower than baseline by 60 min after saline administration on days 2 and 6, but not 10 (day 2: -2.07 ± 0.61 mmol/l, $n=7$, $P<0.01$; day 6: -0.85 ± 0.27 mmol/l, $n=7$, $P<0.05$; day 10: -0.64 ± 0.40 mmol/l, $n=7$, $P>0.05$). Proinsulin concentrations decreased progressively throughout the 60 min sampling period on all three days, but resulted in a significant decrement in concentration at 60 min only on days 6 and 10 (-6.4 ± 2.4 pmol/l and -6.5 ± 2.6 pmol/l, respectively; $n=7$, $P<0.05$ in both cases).

Relationship between plasma insulin and proinsulin concentrations

When all the data for each time period were combined irrespective of treatment, there was a significant linear

relationship between the insulin and proinsulin concentrations at each of the three time periods after birth (Fig. 4). The gradient of this relationship was significantly shallower in period 1 than subsequently (Fig. 4).

Discussion

The results of this study demonstrate that there are changes in the mechanisms of secretion and action of insulin in the foal during the first 10 days of postnatal life. Both insulin and total proinsulin concentrations increased in response to the exogenous administration of glucose and arginine, whereas only the insulin concentration increased significantly when glucose concentrations were increased endogenously by suckling. Basal concentrations of total proinsulin, but not of insulin, also increased over the 10-day period of the study. Furthermore, the gradient of the linear relationship observed between the plasma insulin and proinsulin concentrations was shallower on days 1 and 2 *post partum* than subsequently. These ontogenic changes in the proinsulin concentrations and in the insulin/proinsulin relationship were accompanied by a decrease in the clearance of the exogenously administered glucose.

Insulin concentrations increased in the newborn foal in response to both exogenous and endogenous increases in the circulating glucose concentration. The time course and

magnitude of these responses did not change significantly during the first 10 days of postnatal life and there was no evidence for a neural component to the suckling-induced increase in insulin in the foal, unlike the lamb and rabbit (Bassett & Fletcher 1982). In the current study, the maximum increment in plasma insulin in response to exogenously administered glucose in suckled foals on day 1 was similar to that observed previously in pony foals at 7 days of age and was greater than that seen *in utero* during late gestation or in unsuckled foals 2–3 h after birth (Fowden *et al.* 1982, 1984). These observations suggest that, in foals, the major change in pancreatic β cell sensitivity to glucose occurs with the onset of enteral feeding in the immediate neonatal period. However, the insulin response to glucose at 10 days of age was smaller than that seen in adult horses given a similar intravenous dose of glucose (Garcia & Beech 1986, Giraudet *et al.* 1994). In the foals, the time course and magnitude of the insulin response to arginine also showed no change with postnatal age but, unlike the response to glucose, the insulin response to arginine did not change between late gestation and the neonatal period (Fowden *et al.* 1980, 1984). The neonatal insulin response to arginine was also consistently smaller than that to glucose throughout the 10-day period of the study. Although this may have been in part attributable to the decrease in plasma glucose, overall the current observations indicate that, in common with other species (Hermans *et al.* 1987, Gresores *et al.* 1997), arginine and glucose act through different mechanisms to stimulate the release of insulin from equine β cells. The results also suggest that these two mechanisms are affected differentially by the nutritional adaptations at birth.

The maximum increment in plasma glucose and the insulin response to exogenous glucose were not significantly different on days 1, 5 and 9, but glucose clearance, measured as glucose AUC, was significantly slower on day 1 than subsequently. Hence, foals on day 1 may be insulin resistant and less responsive to the hypoglycaemic action of insulin. High concentrations of hyperglycaemic hormones may be responsible for this insulin antagonism. Noradrenaline concentrations were greater on day 1 than subsequently, and previous studies have shown that cortisol and glucagon concentrations are increased in foals during the first 24 h after birth (Silver *et al.* 1984, Fowden *et al.* 1999). Increased gluconeogenesis may also contribute to the reduced rate of glucose clearance on day 1 as the calorific intake in colostrum is inadequate to meet the energy requirements of the foal in the immediate neonatal period (Ousey *et al.* 1997). Indeed, there were no changes in the plasma glucose concentration after suckling on day 1.

In adult humans, proinsulin concentrations are normally low and increase only in insulin-resistant conditions when the increased demand for insulin leads to the release of immature proinsulin-rich granules from the β cells (Williams *et al.* 1991). High proinsulin concentrations are,

therefore, a useful index of β cell dysfunction and can be used to predict the development of adult-onset metabolic and cardiovascular diseases, such as type 2 (non-insulin-dependent) diabetes and coronary heart disease (Zethelius *et al.* 2002, 2003). In the foals, proinsulin was detected in plasma at all ages studied, although the concentrations were only 5–10% of those of insulin. Basal proinsulin concentrations in the foals on day 1 were similar to those seen in newborn human infants and increased over the next 10 days to reach values seen in normal adult humans during resting conditions (Godfrey *et al.* 1996, Tura *et al.* 2003). In newborn human infants, proinsulin concentrations are inversely correlated to birth weight (Godfrey *et al.* 1996), but no relationships were observed between basal plasma proinsulin and bodyweight of the foals in the current study, either on day 1 or subsequently.

Proinsulin secretion occurred in the foal in response to exogenous administration of glucose and arginine, but not when the increment in glucose was smaller and slower after suckling. Similar proinsulin responses to glucose and arginine have been observed in normal human volunteers (Stumvoll *et al.* 2001, Tura *et al.* 2003). In the foals, the proinsulin responses were smaller and prolonged compared with the corresponding insulin responses throughout the 10-day period of study. These observations are consistent with a relatively small store of proinsulin in the secretory granules and with the lower rate of proinsulin than insulin clearance seen in normal human individuals (Zilker *et al.* 1988, Sobey *et al.* 1989). Proinsulin may, therefore, accumulate in the circulation of the foal as the glucoregulatory demand on the β cells increases with the onset of intermittent provision of nutrient after birth. This could also explain, in part, the increased gradient of the relationship between proinsulin and insulin concentrations seen after the first few days of postnatal life.

In summary, the results demonstrate that equine β cells are responsive to both glucose and arginine during the immediate postnatal period. These secretagogues appeared to act through different mechanisms, and led to the release of insulin and proinsulin. Proinsulin and insulin concentrations were directly related, during both basal and stimulated conditions, although the gradient of this relationship was shallower on days 1 and 2 *post partum* than subsequently. As the secretion of proinsulin in response to glucose and arginine did not change with age of the foals, the increase in proinsulin concentrations over the first 10 days of postnatal life is more likely to reflect the slow clearance rate of proinsulin than a change in the proinsulin content of the secretory granules in the pancreatic β cells.

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