

Insulin and glucose in neonatal calves after peroral insulin and intravenous glucose administration *

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Summary — Effects of peroral insulin on plasma concentrations of immunoreactive insulin (IRI) and glucose in newborn calves were studied. Bovine insulin was administered in amounts of 0.5 mg/kg body weight immediately preceding first colostrum. Thereafter, neither an increased IRI response nor a lowering of blood glucose level were observed, indicating that insulin was either not absorbed from the intestine or possibly retained in the liver. Feeding of whole milk was followed by a higher rise in IRI and glucose concentrations than feeding of colostrum after *po* insulin. However, when compared with 1–3-month old calves, IRI responses to feeding and to iv infused glucose were markedly smaller on the first and second day of life respectively, while glucose responses were similar. This indicates that insulin secretory mechanisms are not fully developed in the newborn calf.

insulin / glucose tolerance / neonate / calf

Résumé — **Insuline et glucose chez le veau nouveau-né après administration orale d'insuline et perfusion intraveineuse de glucose.** *Les effets de l'administration orale d'insuline sur les taux plasmatiques de l'insuline et du glucose ont été étudiés chez le veau nouveau-né. Après l'administration d'insuline précédant immédiatement celle de colostrum, il n'a pas été observé de réponse d'insuline élevée ou de diminution du glucose; ceci indiquerait que l'insuline n'a pas été absorbée par l'intestin ou qu'elle a peut-être été retenue dans le foie. L'insuline et le glucose ont plus augmenté après l'ingestion de lait entier qu'après celle de colostrum. Par rapport à des veaux plus âgés, la réponse de l'insuline au repas ainsi qu'à la perfusion intraveineuse de glucose était nettement inférieure les 2 premiers jours de la vie, tandis que celle du glucose était semblable; ceci indiquerait que les mécanismes sécrétoires de l'insuline chez le veau nouveau-né ne sont pas mis en place.*

insuline / tolérance au glucose / nouveau-né / veau

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INTRODUCTION

The physiology of digestion in the neonate is particular. Thus, in the newborn calf gastric acid secretion is considerably less than in the adult (Pierce *et al*, 1964). Ingested proteins may therefore largely be prevented from degradation. Additionally, the abomasum of preruminant calves selects proteins and peptides that are released to the small intestine. Thus the major part of whey proteins, but not casein, is evacuated rapidly without proteolysis (Yvon *et al*, 1986). Furthermore, during the first hours after birth, high molecular weight proteins, such as immunoglobulins, pass directly through the "open" gut into the circulation of calves. At birth umbilical blood supply is interrupted and nutrients are transported to the liver by the portal vein. However, part of the nutrients absorbed by the digestive tract as well as pancreatic hormones flowing into the portal vein may by-pass the liver and reach the general circulation directly through the arterial canal, which is not suddenly occluded (Botti *et al*, 1982).

There are high amounts of insulin in bovine colostrum (Ballard *et al*, 1982; Slebodzinski *et al*, 1986; Malven *et al*, 1987; Ronge and Blum, 1988; Collier *et al*, 1989; Wahab *et al*, 1989; Grütter and Blum, unpublished data), which rapidly decrease to the low levels present in milk during later stages of lactation. However, only few investigations have been carried out on insulin in newborn calves (Young *et al*, 1970; Breier *et al*, 1988; Ronge and Blum, 1988; Oda *et al*, 1989). Pierce *et al* (1964) found increased bioactive serum insulin and a lowering of blood glucose levels after *po* administration of high amounts of bovine insulin to newborn calves, suggesting that insulin may be absorbed from the gut of the newborn calf.

The primary objective of our investigations was to study effects of *po* administered insulin preceding feeding of first colostrum, in comparison with feeding of whole milk, on plasma concentrations of immunoreactive insulin (IRI) and glucose in newborn calves. In addition, responses of IRI and glucose to *iv* infused glucose were studied in blood plasma of 2-d-old calves compared with older calves.

MATERIALS AND METHODS

Experimental procedures

Experiment 1

Eight eutocic newborn calves (2 males and 6 females; 4 Simmental crossbreds (SiRH), 3 Swiss Brown (SB) and 1 SB x SiRH) were studied. They were born at term and received their first meal (maternal colostrum) in the morning (between 08.15 and 09.00 h), at 4.5 h (h) after birth (average). Thereafter, until the end of the experiments colostrum in amounts of \approx 5% of body weight (BW) was offered twice daily (between 05.30 and 07.30 h, 16.00 and 18.00 h). Calves were *po* given 0.5 mg insulin/kg BW immediately preceding the first colostrum (5%—one calf 4%—of BW). Bovine insulin was obtained from Sigma Chemical Company, St Louis, MO, USA (No 1-5500). Activities ranged from 23.4–26.8 IU/mg. Insulin was dissolved (1:1 000) in 0.9% saline containing 0.1% bovine gammaglobulin (final pH 7.4).

Three additional eutocic newborn calves (males, SiRH) served as controls. They were born at term and received their first meal in the morning (between 07.45 and 08.30 h), at 5.5 h after birth (average). They were given whole milk in amounts of 5% of their BW during the first 2 feedings, while the first colostrum was preserved in a refrigerator up to the third meal (5% of BW). After the first meal, time of feedings was similar to that of group A.

Blood samples (10 ml, A; or 40 ml, B) of these calves were taken at 0.25 h before and at

0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 8 h after the first meal. In group A, additional blood samples were taken at 3.5, 4, 5, 6, and 7 h after the first meal.

Experiment 2

On the second day (d) of life, starting at 10.00 h, an iv glucose tolerance test was performed: 0.33 g glucose/kg^{0.75} BW x min was infused for 5 min in 7 of the 8 calves.

Ten 1–3-month-old milk-fed male or female calves (SB or Red Holstein) served as controls. They received milk in amounts of 3–7 l between 07.00 and 08.00 h. Calves were iv infused with 0.33 g/kg^{0.75} BW x min for 5 min, starting at 6 h after feeding.

Blood samples (10 ml) from these calves were taken immediately before and at 5, 10, 15, 20, 30, 45, 60 min after the start of infusion.

Blood samples were taken from the external jugular vein, either with vacutainers containing lithium heparin (Monoject, Sherwood Medical, St Louis, MO, USA) (exp 1) or through indwelling catheters, implanted in calves prior to the first feeding (exp 2A), on the evening before the experiment (exp 2B). Blood from catheters was transferred to polystyrene tubes containing lithium heparin (HEP-95, Milian Instruments SA, CH-1228 Plan-les-Ouates/Geneva). Tubes were centrifuged at 1 500 g and 4 °C for 15 min. Plasma was then partitioned into aliquots of ≈ 1.5 ml and stored in polypropylene microtubes (Milian, Geneva) at –18 °C until analyzed for IRI and glucose.

All animals were held at the Federal Research Station for Animal Production, Grange-neuve/CH-1725 Posieux.

Laboratory methods

Blood plasma concentrations of IRI and glucose were measured as recently described (Zimmerli and Blum, 1990).

Statistical analysis

Data are expressed as means ± SEM. Total responses of IRI and glucose were evaluated by

calculation of areas under concentration curves after subtraction of (mean) basal levels (Δ 0–3 h and Δ 0–8 h, without consideration of values at 4, 5, 6 and 7 h; Δ 0–60 min, respectively). Half-time ($T_{1/2}$) and clearance rate (K) of glucose in iv glucose tolerance tests were calculated according to Kaneko (1980). The significance of changes after treatments within groups was evaluated by Wilcoxon Signed Ranks test. The significance of differences between groups (net changes (Δ) of single values and total responses) was evaluated by the Mann–Whitney U test, using the SYSTAT program (SYSTAT Inc, Evanston, IL, USA). In exp 1, Pearson's correlation coefficient between total responses of IRI and glucose (Δ 0–8) was computed using the SAS program (SAS Institute Inc, Cary, NC, USA).

RESULTS

Concentrations of IRI and glucose in newborn calves after peroral administration of insulin or ingestion of whole milk (exp 1, fig 1)

Concentration of IRI and glucose increased significantly after *po* administration of insulin, which immediately preceded first colostrum intake ($P \leq 0.05$). In calves fed whole milk (but no additional insulin), increases of IRI and glucose concentrations were much more pronounced than in calves fed insulin preceding colostrum. Changes (Δ) in glucose concentration at 1.5, 1.75, 2, 2.5 and 3 h were significantly smaller in calves fed insulin preceding colostrum than in calves fed whole milk ($P \leq 0.05$). Likewise, total responses of IRI (Δ 0–8 h) and glucose (Δ 0–3 h and Δ 0–8 h) respectively were significantly smaller in calves fed insulin preceding colostrum than in calves fed whole milk ($P \leq 0.05$). There was a positive correlation between total responses of IRI and glucose (Δ 0–8 h) in calves fed colostrum or whole milk ($r = 0.56$; $P = 0.07$).

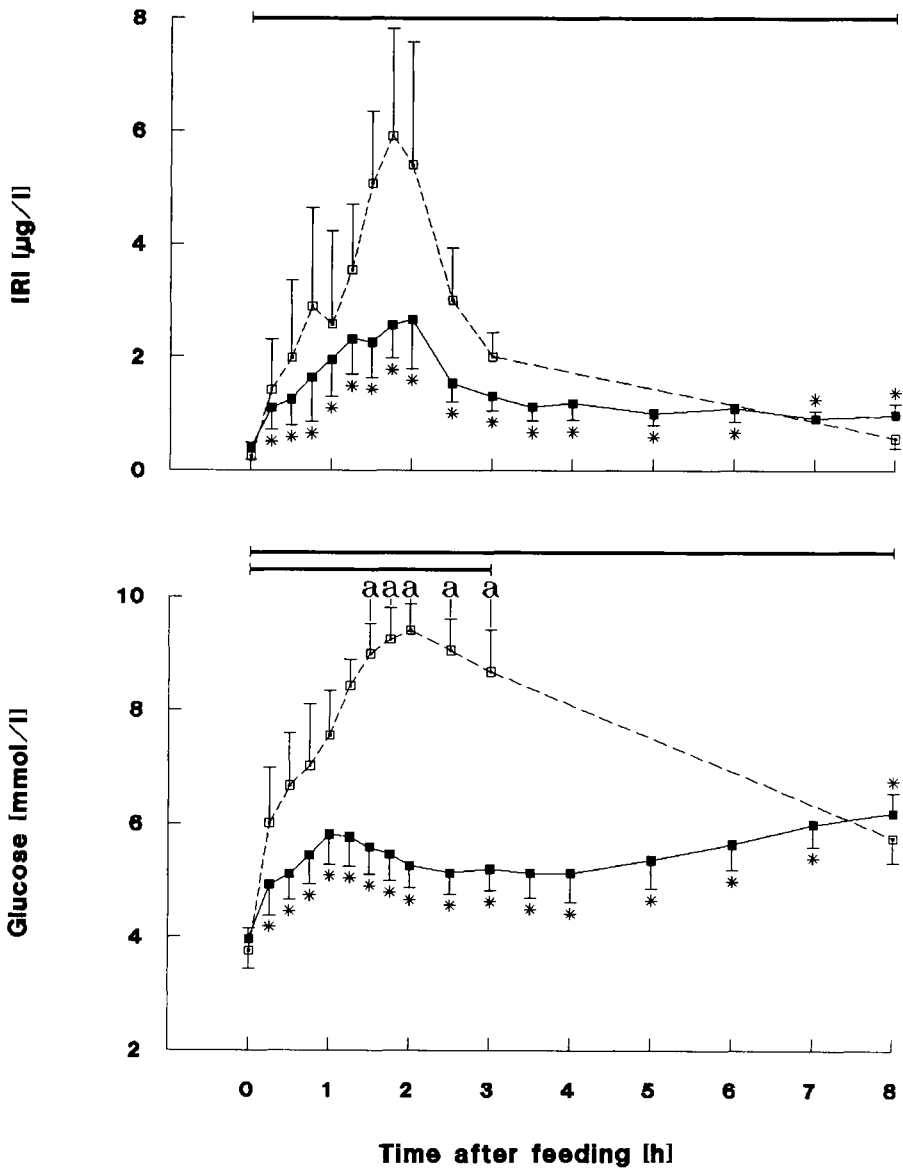


Fig 1. Plasma concentrations of insulin and glucose in newborn calves before (0 h) and for 8 h after either the po administration of 0.5 mg insulin/kg BW preceding the ingestion of first colostrum (—, filled symbols) or the ingestion of whole milk (---, open symbols). * Significantly ($P \leq 0.05$) different from basal level (0 h); a significantly ($P \leq 0.05$) different from response to feeding of whole milk; — significantly ($P \leq 0.05$) different total responses ($\Delta 0-3$ h, $\Delta 0-8$ h) between calves fed colostrum and those fed whole milk (without consideration of values at 4, 5, 6 and 7 h).

Effects of intravenous glucose on concentrations of IRI and glucose in calves on the second day of life or at the age fo 1–3 months (exp 2, fig 2)

Basal concentrations of IRI were 0.68 ± 0.14 and 0.36 ± 0.06 $\mu\text{g/l}$ in 2-d-old and older calves, respectively. Concentration of IRI increased immediately after the start of the infusion and peaked at 20 min in both 2-d-old and older calves. All values were significantly higher than basal levels ($P \leq 0.05$). Changes in IRI concentration (Δ) were consistently smaller in 2-d-old than in older calves ($P \leq 0.05$ at 30 min and $P \leq 0.1$ at 5 and 45 min). Total IRI response (Δ 0–60 min) tended to be smaller in 2-d-old than in older calves ($P = 0.051$).

Basal concentration of glucose was significantly higher in 2-d-old than in older calves (8.02 ± 0.29 and 4.67 ± 0.13 mmol/l, respectively; $P \leq 0.001$). Glucose concentration increased sharply after the start of the infusion and was highest at 5 min in both 2-d-old and older calves. Thereafter, glucose concentration decreased in a similar manner in 2-d-old and older calves ($T_{1/2} = 31.2$ and 29.5 min; k values = 2.2 and 2.5%/min respectively) and almost reached basal levels after 60 min. Values from 5–45 min were significantly higher than basal levels ($P \leq 0.05$). Changes in glucose concentration (Δ) tended to be smaller at 10 min, but higher at 60 min in 2-d-old than in older calves ($P \leq 0.1$).

DISCUSSION

Pierce *et al* (1964) found increased bioactive serum insulin and a lowering of blood glucose levels after *po* administration of bovine insulin to newborn calves, especial-

ly if administered in alkaline solution. Contrary to our study, glucose was usually fed prior to insulin administration. In addition, insulin-like activity was determined by an indirect biological method and not radioimmunologically as in our study. As in neonatal calves, serum concentrations of colostrum small proteins increased more rapidly after the first feeding than those of IgG (Kiryama *et al*, 1989), a similar rapid increase of plasma IRI levels was expected in our calves, also. In contrast, neither an increased IRI response nor a lowering of blood glucose level were observed after *po* administration of comparably excessive amounts of insulin (≈ 500 times the quantity which was measured in 2 l of first colostrum; Grütter and Blum, unpublished data) preceding first colostrum. The reasons for the discrepancy between our data and those of Pierce *et al* (1964) are not clear. Feeding of colostrum, which immediately followed insulin administration, may have prevented insulin from absorption by inclusion in the food matrix. However, in 3 unfed newborn calves (not shown), administration of insulin in buffered solution alone or together with lactose did not influence plasma concentrations of IRI and glucose. This, too, was not the case, if insulin administration was immediately preceded by the injection of lysine-vasopressin, shown to facilitate contraction of the oesophageal groove in cattle (Scholz and Mikhail, 1987; van Weeren-Keverling Buisman *et al*, 1990), thus favouring a direct passage of ingested insulin into the abomasum. Whether uptake by the liver during the first passage possibly prevents absorbed insulin from appearance in the peripheral blood still requires investigation.

Amounts of lactose were considerably lower in first colostrum than in pooled milk (morning/evening) taken on the 7th or 12th

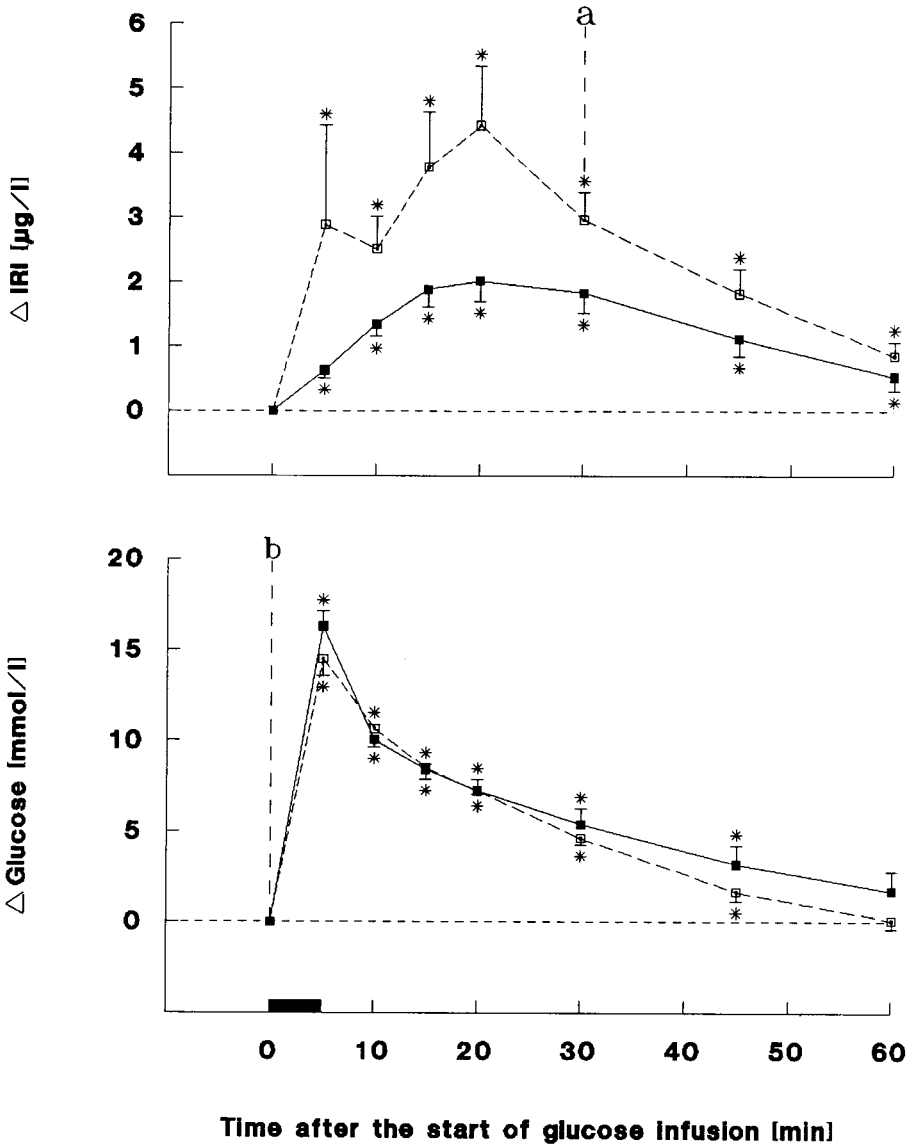


Fig 2. Intravenous glucose tolerance test: changes in plasma concentrations (Δ) of insulin and glucose in calves before (0 min) and for 60 min after the start of glucose infusion (—; 0.33 g/kg^{0.75} BW x min for 5 min) on the 2nd day of life (—, filled symbols) or at 1–3 months of age (---, open symbols). * Significantly ($P \leq 0.05$) different from basal level; a significantly ($P \leq 0.05$) different from response in 1–3-month old calves; b significantly ($P \leq 0.001$) different from basal level in 1–3-month-old calves.

d after parturition (35.3 ± 2.0 vs 49.2 ± 1.0 g/l) (not shown). Therefore, the significantly higher responses of IRI and glucose to feeding of whole milk when compared with colostrum after *po* insulin were probably due to the higher lactose content of whole milk. Thus, the higher rise in blood glucose level after ingestion of whole milk may have caused the increased IRI response. In agreement, we found a positive correlation between postprandial responses of IRI and glucose in newborn calves fed colostrum or whole milk. On the other hand, the lower IRI response in calves fed colostrum following *po* insulin than in those fed whole milk could reflect suppression of endogenous insulin secretion by ingested exogenous insulin or other colostrum constituents. Thus, insulin-like growth factor I, which is present in colostrum in high amounts (Francis *et al*, 1986; Ballard *et al*, 1987; Malven *et al*, 1987; Francis *et al*, 1988; Ronge and Blum, 1988; Campbell and Baumrucker, 1989; Collier *et al*, 1989; Oda *et al*, 1989; Vega *et al*, 1989; Grütter and Blum, unpublished data), was shown to inhibit insulin secretion in man (Guler *et al*, 1989). However, it remains to be clearly demonstrated that insulin-like growth factor I can be absorbed from gut of the newborn calf as a precondition to suppress insulin secretion. Alternatively, other blood glucose regulating hormones, such as glucagon, glucocorticoids and catecholamines could have been released in different amounts in response to the ingestion of first colostrum following *po* insulin or whole milk and, in consequence, could have influenced postprandial blood glucose and IRI levels.

Plasma insulin level in \approx 3-month-old milk-fed calves rose to $16 \mu\text{g/l}$ after feeding (Kamalu and Trenkle, 1978). At the same time, a high plasma glucose concentration was measured. Feeding of whole milk was followed by an increase of blood glucose in

the range of that observed by Kamalu and Trenkle (1978) in our newborn calves too, but IRI concentration only rose to $5.92 \mu\text{g/l}$, which is also considerably less than what was observed in 2–3-month-old milk-fed calves (Zimmerli and Blum, 1990). This indicates that insulin secretory mechanisms are not fully developed in the newborn calf.

The duration of insulin-induced hypoglycaemia in calves was prolonged immediately after birth, indicating that the factors normalizing blood glucose concentration are not very efficient at this time (Comline and Edwards, 1968). Therefore in the newborn the sensitivity of the liver to insulin may be low (Demigné and Rémésy, 1984). In our study, the IRI response to *iv* glucose was markedly smaller in 2-d-old than in older calves, whereas changes in glucose concentrations were similar in both groups. Glucose utilization is known to decrease with age (Young *et al*, 1970), mainly as a result of transition from non-ruminant to ruminant metabolism. However, it remains to be shown whether different utilization rates in 2-d-old to those in 1–3-month-old calves could have significantly influenced our data. Higher basal levels of blood glucose in 2-d-old than in older calves might, in addition, be due to differing time relative to feeding.

CONCLUSION

In conclusion, the results suggest that exogenous insulin ingested with maternal colostrum may not substantially contribute to circulating IRI levels, for it did not appear to be absorbed from the gut or, if absorbed, obviously did not reach the general circulation in significant amounts. In addition, insulin secretory mechanisms do not seem to be fully developed in the newborn calf.

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