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Differences between Lactating and Non-Lactating Dairy Cows in Concentration and Secretion Rate of Insulin

By MICHAEL A. LOMAX,* G. DAVID BAIRD, CHRISTOPHER B. MALLINSON and HERBERT W. SYMONDS
Agricultural Research Council Institute for Research on Animal Diseases, Compton, Berkshire RG16 0NN, U.K.

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1. Four parameters of insulin metabolism were compared in catheterized lactating and non-lactating Friesian × Ayrshire dairy cows. 2. The four parameters, i.e. arterial and portal-venous concentrations of insulin, and pancreatic output and hepatic uptake of insulin, were approx. 2-, 3-, 3- and 5-fold higher respectively in the non-lactating cows than in the lactating cows in the normal fed state. Statistical significance was not achieved for the differences in magnitude in the case of the latter two parameters, however. 3. All four parameters increased significantly about 4-fold when non-lactating cows were infused intravenously with glucose for 48h at a rate of 4.2mmol/min. The parameters also increased in the lactating cows during glucose infusion, but the values reached were substantially lower than in the non-lactating cows and the increases were not statistically significant. 4. Arterial insulin concentrations doubled in the non-lactating cows during a 3h infusion of propionate into a mesenteric vein, but remained unaltered in the lactating cows. 5. Differences in insulin concentration and output between the lactating and non-lactating cows were not consistently related to differences in either glucose concentration or glucose-entry rate. Arterial propionate concentrations were similar in both groups of cows at all times. 6. It is concluded that in the dairy cow, insulin secretion in response to an insulinotropic agent is diminished during lactation.

There have been several reports in recent years that in the cow there is an inverse relationship between blood insulin concentration and milk yield. Thus during lactation in the dairy cow, blood insulin concentrations are lowest at about 2 to 4 weeks after calving when milk yield is at its peak. Subsequently, insulin concentrations begin to rise as lactation progresses and milk yield declines (Koprowski & Tucker, 1973; Jenny *et al.*, 1974; Smith *et al.*, 1976). Blood insulin concentrations are also lower throughout lactation in high-milk-yielding dairy cows than in low-milk-yielding beef cows (Hart *et al.*, 1978). Furthermore the rise in blood insulin concentration in response to feeding was greater in the lactating beef cows than in the lactating dairy cows (Hart *et al.*, 1975). The differences in insulin concentration and response observed by Hart *et al.* (1975, 1978) could possibly have been genetically determined, since different breeds of cows were compared. This criticism cannot, however, be made of the other studies demonstrating an inverse relationship between insulin concentration and milk yield during the course of a given lactation.

The association of low insulin concentration with maximal lactation could simply be a consequence of the fact that high-milk-yielding dairy cows are usually in a state of negative nutritional balance at this time. The low insulin concentration would then be of value in allowing mobilization of endogenous energy sources in the form of amino acids and non-esterified fatty acids. However, poor correlations between blood insulin and blood glucose concentrations have led Smith *et al.* (1976) to suggest that negative energy balance is not the only factor involved.

In connection with the use of appropriately catheterized lactating and non-lactating dairy cows of the same breed to study net metabolism across the gut and liver *in vivo*, it was observed that the insulin response to variation in nutrient supply differed between the lactating and non-lactating cows. The present paper deals with the differences that were observed. The results indicate that in the dairy cow there is a diminished rate of insulin secretion during lactation. A preliminary report of some of the findings has been published (Lomax *et al.*, 1978).

Experimental

Materials

p-Aminohippuric acid was obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K., propionic acid (99% pure) was from

* Present address: Biological Laboratory, The University, Canterbury, Kent CT2 7NJ, U.K.

Koch–Light Laboratories, Colnbrook, Bucks., U.K. and glucose (AnalaR) was from BDH Chemicals, Poole, Dorset, U.K. Double-distilled water, the second distillation being from glass, was used throughout.

Animals

Nine mature non-pregnant Friesian × Ayrshire dairy cows of similar weight were used, each of which had calved on at least two occasions. At the time of experiment, five of the cows were lactating and four were non-lactating. All the animals had been catheterized surgically for the measurement of portal and hepatic metabolite-production rates by a combination of the methods of Symonds & Baird (1973) and Baird *et al.* (1975). The catheterization involved placing catheters in a mesenteric vein, the portal vein at the *porta hepatis*, a hepatic vein and a carotid artery in each cow. The animals were allowed to recover from surgery for at least 3 weeks, and to return to normal feeding, before being used in the experiments. When feeding normally, the non-lactating cows were receiving about 5kg of medium-quality hay and 3kg of dairy concentrate per day, and the lactating cows were receiving in addition 0.3kg of dairy concentrate/kg of milk produced. The daily feed was given in two equal portions, the first at about 07:30h and the second at about 14:30h. At the time of glucose infusion, the three lactating cows were in their second or third month of lactation and giving an average milk yield of 17.3kg/day. The average daily intake of metabolizable energy for each animal was calculated to be about 122MJ in the lactating cows and 66MJ in the non-lactating cows. These intakes provided about 93 and 122% of (requirement+safety margin) for the two groups of cows respectively according to Technical Bulletin 33 (1975).

Experimental procedure

Insulin status was compared in the lactating and non-lactating cows in the following situations: (1) the normal fed state; (2) before, during and after (*a*) infusion of glucose and (*b*) infusion of propionate. Arterial concentrations of insulin were measured in all three situations. Values for portal and hepatic production rates of insulin were obtained only in situations (1) and (2*a*), however.

(1) *Normal state.* Blood-flow rate was measured by the infusion of sodium *p*-aminohippurate into the catheterized mesenteric vein (see Snoswell *et al.*, 1978). The infusion was begun at about 10:00h to 10:30h by using a Harvard infusion–withdrawal syringe pump (Harvard Apparatus, Millis, MA, U.S.A., obtained from T.E.M. Sales, Crawley, Sussex, U.K.). Sets of samples of blood from the carotid artery, the portal vein and the hepatic vein were then taken between 11:30h and 12:30h for measurement of the concentrations of blood metabolites. Portal and hepatic plasma-flow rates were calculated from the plasma *p*-aminohippurate concentrations, and insulin-production rates were calculated from these values for flow rate and from the appropriate plasma-insulin concentrations (see Katz & Bergman, 1969). Production rates of glucose were calculated in a similar manner, but with whole-blood-flow rates, obtained by multiplying plasma-flow rates by 1/(1−haematocrit value). Several of the normal-state observations were in fact observations made immediately before infusion of glucose or propionate (see below).

(2*a*) *Glucose infusion.* The experiment was started by following the procedure described in (1) above. Immediately after removal of the blood samples, the infusion of *p*-aminohippurate was stopped and infusion of an aqueous solution of 2.8M-glucose was begun at a constant rate of 1.5ml (4.2mmol)/min, i.e. about 1.5mg/kg body wt. per min, via a catheter placed in a jugular vein at least 48h previously. The infusion was performed by using a Harvard Lambda pump as previously described (Treacher *et al.*, 1976). Further blood samples were then taken at 4.5, 24 and 48h after starting the glucose infusion. The procedure after 24 and 48h of infusion was as described in (1) above, except that glucose was being infused. For the measurement after 4.5h of infusion, blood samples were taken at about 16:30h, and infusion of *p*-aminohippurate was consequently begun at about 15:00h. After the 48h samples had been taken, the glucose infusion was stopped and the procedure described in (1) above was carried out 24h later, i.e. at 72h after the start of glucose infusion. The animals were allowed to eat normally during the glucose infusion and the infusion did not appear to affect appetite. Control infusions were run in which water replaced the glucose solution. Of the lactating and non-lactating cows, three of each group were infused with glucose and two of each group were infused with water.

(2*b*) *Propionate infusion.* Infusion of *p*-aminohippurate was maintained throughout this experiment. The experiment was started by following the procedure described in (1) above. Subsequently, an aqueous solution of 5.0M-sodium propionate was infused for 3h at a constant rate of 2.0ml (10mmol)/min via the mesenteric catheter by using the Harvard Lambda pump. Blood samples for the measurement of blood-flow rate and blood-metabolite concentrations were then taken after the propionate infusion had been in progress for 0.5, 1.5 and 3.0h, and then again 1.5h after the propionate infusion had ceased, i.e. at 4.5h after starting the propionate infusion. The afternoon feed was not given until the propionate infusion had been stopped, i.e. between 14:30h and

15:30h. Control infusions were run in which 5.0M-NaCl replaced the sodium propionate. Of the lactating and non-lactating cows, three of each group were infused with propionate and two of each group with NaCl.

Blood

Blood samples were removed by syringe via the catheters implanted in the carotid artery, the portal vein and the hepatic vein, and collected into heparin at 0°C for the determination of insulin and *p*-aminohippurate, and into 6% (w/v) $HClO_4$ for the determination of glucose, acetate, D-3-hydroxybutyrate and propionate (Baird & Heitzman, 1970). The heparin-treated blood was centrifuged for 15min at 1500*g* within 2h of collection and the plasma removed and stored frozen before being assayed. Insulin concentration in the plasma samples was determined by radioimmunoassay by using the radioimmunoassay kit supplied by The Radiochemical Centre, Amersham, Bucks., U.K. Although this kit is designed for the assay of insulin in human plasma, it gave results with standard bovine insulin that were essentially identical with those obtained with standard human insulin. Non-specific binding of radioactivity amounted to 4.6% on average, and mean recovery of insulin added to bovine plasma was 95.9% over a concentration range of 25–150m-units of insulin/litre. The concentration of *p*-aminohippurate was determined by the method of Harvey & Brothers (1962). Glucose concentration in the neutralized $HClO_4$ extracts of whole blood was determined by the method of Bergmeyer *et al.* (1974), acetate concentration by the method of Snoswell *et al.* (1978) and D-3-hydroxybutyrate concentration by the method of Williamson & Mellanby (1974). Propionate concentration was also determined in these extracts, after acidification, in a Pye–Unicam 204 gas-liquid chromatograph (Pye–Unicam, Cambridge, U.K.). The column used was that employed by Baird *et al.* (1975).

Control of pyrogenicity

To avoid eliciting a pyrogenic response in the cows during infusions, all liquids to be infused were passed at least once, under pressure, through an appropriate filter held in an autoclaved sterilizing stainless 142mm filter holder [Millipore (U.K.) Ltd., London NW10 7SP, U.K.]. Solutions of *p*-aminohippurate were routinely filtered twice in this manner. Immediately before use, infusion pumps and lines were washed out with a dilute hypocholorite solution [Parazone; Jeyes (U.K.) Ltd., Thetford, Norfolk, U.K.] and with a chemical sterilizing fluid (Novasapa; Pharmaceutical Manufacturing Co., Bolton, Lancs., U.K.), followed by sterile iso-osmotic saline [0.9% (w/v) NaCl], and finally the solution to be infused.

Statistics

The significances of differences in insulin concentrations and production rates between the normal fed lactating and non-lactating cows were determined by Student's *t* test. For each of the two infusion experiments a 2×2 factorial split-plot-in-time analysis of variance was performed on the data, with treatment (i.e. experimental or control) and lactation groups as main-plot effects, and time of sampling as a sub-plot effect.

Results

Table 1 compares parameters of insulin metabolism that were measured in the lactating and non-lactating cows when the animals were in the fed untreated state. Both the arterial and portal concentrations of insulin were more than twice as high in the non-lactating cows as in the lactating cows, and the differences between the two groups were statistically significant. The portal production rate of insulin is probably the same as the rate of secretion of insulin from the pancreas, since the pancreatic veins are tributaries of the portal vein (Sisson & Grossman,

Table 1. *Parameters of insulin metabolism in the lactating and non-lactating cows in the fed untreated state*
The values are in each case the means for four cows in each group, except for the arterial concentration in the lactating group, which is for five cows. For each parameter, the value for a given cow was in turn derived by obtaining the mean for all the values that had been obtained for that animal on different days. **, $P<0.01$; ***, $P<0.001$ (compared with the lactating group). $kg^{0.75}$ is equivalent to 1 kg of metabolic body weight.

Parameter	Units	Lactating cows	Non-lactating cows	Standard error of difference between means
Arterial concentration	m-units/litre	14.5	32.3***	2.7
Portal concentration	m-units/litre	17.4	46.4**	6.1
Portal production rate	m-units/min	50.5	174.7	54.1
Hepatic production rate	m-units/min	−29.9	−145.3	58.3
Portal production rate	m-units/h per/$kg^{0.75}$	30.1	92.7	25.8
Hepatic production rate	m-units/h per/$kg^{0.75}$	−18.0	−77.6	30.2

1938), and in the sheep, at least, there is little or no uptake of insulin by the gut (Brockman & Bergman, 1975). As Table 1 shows, portal production, and hence pancreatic output, of insulin was over 3 times as great in the non-lactating cows as in the lactating cows, although the difference was not statistically significant. Correspondingly, hepatic uptake was also greater in the non-lactating cows, although again the difference was not significant. Similar differences were obtained when the production rates were related to metabolic weight. The means for all the individual values for portal production rate of insulin that were obtained for the lactating and non-lactating groups of cows on separate days in the fed untreated state are 39.4 ± 12.4 (mean $\pm$ S.E.M.; $n = 10$) and 141.9 ± 23.2 (mean $\pm$ S.E.M.; $n = 13$) respectively. These means are significantly different ($P < 0.01$).

Tables 2 and 3 give the plasma concentrations and production rates of insulin respectively that were obtained in the lactating and non-lactating groups of cows before, during and after intrajugular infusion with either glucose or water. In the non-lactating cows (Table 2) glucose infusion caused 4-fold increases in arterial and portal insulin concentrations that persisted throughout the infusion. These increases were highly significant. By contrast, the glucose infusion had much less effect in the lactating cows. In this latter group, arterial insulin concentration had only doubled and portal insulin concentration trebled after 4.5h of infusion, and the concentration increases in this case were not statistically significant. Furthermore, after 24h of glucose infusion both the arterial and portal insulin concentrations had returned to values that were similar to the pre-infusion values. The water infusions had no effect on arterial or portal insulin concentrations in either group of cows. Table 3(*a*) shows that in line with its effects on arterial and portal concentrations of

Table 2. *Effect of infusion of glucose on arterial and portal-vein concentrations of insulin in lactating and non-lactating cows*
For details of infusion procedure, see the Experimental section. ***, $P < 0.001$ (compared with pre-infusion value).

		Insulin concn. (m-units/litre)					
			Time elapsed during infusion				
Cow status	Treatment	Pre-infusion	4.5h	24h	48h	Post-infusion	Standard error of difference between means
(*a*) Arterial concentration							
Lactating	Glucose-infused	16.5	36.2	17.7	18.9	15.1	18.6
	Control	13.2	17.3	16.7	16.8	15.1	22.8
Non-lactating	Glucose-infused	37.0	130.1***	145.6***	145.7***	47.8	18.6
	Control	25.9	26.3	30.0	43.6	31.6	22.8
(*b*) Portal concentration							
Lactating	Glucose-infused	18.9	53.7	27.4	23.2	17.1	30.5
	Control	23.2	28.1	22.8	24.8	24.4	52.8
Non-lactating	Glucose-infused	52.2	176.6***	223.5***	203.8***	62.0	30.5
	Control	42.6	45.9	44.8	53.9	43.6	43.1

Table 3. *Effect of infusion of glucose on portal and hepatic production rates of insulin in lactating and non-lactating cows*
For details of infusion procedure, see the Experimental section. *, $P < 0.05$; **, $P < 0.01$ (compared with pre-infusion value).

		Insulin-production rate (m-units/min)					
			Time elapsed during infusion				
Cow status	Treatment	Pre-infusion	4.5h	24h	48h	Post-infusion	Standard error of difference between means
(*a*) Portal production							
Lactating	Glucose-infused	23.8	247.7	89.0	101.7	47.0	214.7
	Control	48.0	113.4	49.1	49.0	64.2	371.8
Non-lactating	Glucose-infused	198.2	553.7	947.8**	850.1*	201.6	214.7
	Control	142.7	212.0	170.0	111.8	147.0	371.8
(*b*) Hepatic production							
Lactating	Glucose-infused	−67	−166	−10	−6	−115	194
	Control	−98	−110	−90	−43	2	336
Non-lactating	Glucose-infused	−105	−532*	−361	−499	−100	194
	Control	−69	59	−142	−121	151	336

insulin, the glucose infusion caused a much larger increase in portal output of insulin in the non-lactating cows than in the lactating cows. In the non-lactating cows, glucose infusion elicited a 2.5–4-fold increase in portal output of insulin that persisted throughout the infusion period and was statistically significant at 24h and 48h of infusion. The glucose infusion also appeared to produce an effect in the lactating cows, since portal output after 4.5h of infusion was 10 times greater than that before infusion. Nevertheless, this increase was not significant and the value reached was only of a similar order to that observed in the non-lactating cows before infusion. As with the values for insulin concentration, portal production of insulin appeared to decline in the lactating cows as the glucose infusion progressed. Finally, Table 3(*b*) shows that in the non-lactating cows hepatic uptake of insulin increased in step with the increase in portal output, and was between 3 and 5 times greater than the pre-infusion value at all measurement times during the glucose infusion. Significance was only achieved for the increase at 4.5h, however. By contrast, the glucose infusion had no clear effect on hepatic uptake of insulin in the lactating cows.

The effect of the intramesenteric infusion of propionate on arterial concentrations of insulin is recorded in Table 4. The propionate infusion did not cause any change in the lactating cows. In the non-lactating cows, on the other hand, infusion elicited a significant 2-fold increase in insulin concentration that persisted over the first 1.5h of the infusion. The insulin concentration in the non-lactating cows had, however, returned to the pre-infusion value by the time the propionate infusion had been in progress for 3h.

Table 5 records the effect of the glucose and propionate infusions on the arterial concentrations of glucose and propionate. The glucose infusion caused significant increases in glucose concentration in both the lactating and non-lactating cows, and the highest concentration that was reached was in the

Table 4. *Effect of propionate infusion on the arterial concentration of insulin in lactating and non-lactating cows*
For details of the infusion procedure, see the Experimental section. *, $P<0.02$; **, $P<0.01$ (compared with pre-infusion value).

		Insulin concn. (m-units/litre)					
			Time elapsed during infusion				
Cow status	Treatment	Pre-infusion	0.5h	1.5h	3.0h	Post-infusion	Standard error of difference between means
Lactating	Propionate-infused	12.4	15.2	14.6	17.1	13.3	17.2
	Control	12.3	11.1	12.2	11.0	14.3	21.0
Non-lactating	Propionate-infused	45.7	110.1**	94.4*	47.5	39.7	17.2
	Control	42.0	26.4	33.6	28.3	40.2	21.0

Table 5. *Effect of the glucose and propionate infusions on arterial concentrations of glucose and propionate in the lactating and non-lactating cows*
For details of infusion procedures, see the Experimental section. *, $P<0.05$; ***, $P<0.001$ (compared with pre-infusion value). Results for the control animals have been omitted for clarity. However, these results were used in the statistical analysis.

		Metabolite concn. in whole blood (mM)					
			Time elapsed during infusion				
Metabolite	Cow status	Pre-infusion	4.5h	24h	48h	Post-infusion	Standard error of difference between means
(*a*) Glucose infusion							
Glucose	Lactating	2.86	4.26***	3.42*	3.27	2.72	0.24
	Non-lactating	3.25	3.91*	3.81*	3.87*	3.31	0.24
Propionate	Lactating	0.03	0.03	0.03	0.04	0.04	0.01
	Non-lactating	0.03	0.04	0.03	0.05*	0.03	0.01
			Time elapsed during infusion				
			0.5h	1.5h	3.0h		
(*b*) Propionate infusion							
Glucose	Lactating	2.62	2.88*	2.99*	2.98*	3.03*	0.13
	Non-lactating	3.34	3.44	3.55	3.32	3.44	0.13
Propionate	Lactating	0.04	0.15***	0.15***	0.14***	0.03	0.02
	Non-lactating	0.03	0.14***	0.11***	0.14***	0.06	0.02

lactating cows after 4.5h of infusion. Glucose infusion did not have any consistent effect on propionate concentrations. The propionate infusion also increased glucose concentrations in both groups of cows, although statistical significance was only obtained for the increases in the lactating cows. The glucose concentration in the lactating cows did not, however, reach that in the non-lactating cows at any stage of the propionate infusion. During propionate infusion, arterial propionate concentrations increased to a similar extent in both groups, and these increases were statistically significant.

In ruminants, hepatic output of glucose normally accounts for about 85% of glucose-entry rate (Bergman, 1973), and can consequently be taken as a rough approximation of this parameter. In animals infused with glucose, glucose-entry rate would then be the sum of hepatic glucose output and the rate of infusion of glucose (i.e. 4.2mmol/min). The effects of the various infusions on glucose-entry rate measured in this way are recorded in Table 6. Before infusion, entry rate, i.e. hepatic glucose output alone, was about twice as high in the lactating cows as in the non-lactating cows. Glucose infusion caused little change in entry rate in the lactating cows, since hepatic output of glucose decreased by a value approximately equal to the rate of infusion of glucose. By contrast, there was little or no decrease in hepatic glucose output in the non-lactating cows during the glucose infusion, except non-significantly at 24h, and consequently total glucose entry rate increased to values of the same order as those seen in the lactating cows. A similar picture emerged from the propionate infusions. Here, glucose entry rate remained unchanged in the lactating cows for 1.5h of propionate infusion, at least. In the non-lactating group, however, propionate infusion increased glucose entry to values otherwise characteristic of lactating cows and these increased values were maintained throughout the infusion.

Discussion

In view of the negative correlation between blood insulin concentration and milk yield (Koprowski & Tucker, 1973; Jenny *et al.*, 1974; Smith *et al.*, 1976), it might seem logical that arterial blood insulin concentrations would be higher in non-lactating cows than in lactating cows of the same breed, as demonstrated in Table 1. However, Hart *et al.* (1978) found no clear difference in insulin concentration in cows at maximal lactation and in the same animals when they had ceased to lactate. The difference may arise because the insulin results of Hart and co-workers were mean values obtained from hourly blood samples taken over 24h, whereas in the present study the insulin values were measured at a constant time after feeding. Hart *et al.* (1978) also used diets containing a high proportion of concentrate feed. Such diets tend to elicit relatively high concentrations of circulating insulin (Walker & Elliot, 1973), and this in turn could have obscured differences due to lactation. In the present study, the decrease in circulating concentrations of insulin during lactation appeared to be due to a decrease in pancreatic output of insulin, rather than to any increase in hepatic uptake. On average, the liver took up some 60% of the pancreatic output of insulin in the lactating cows and 85% in the

Table 6. *Effect of the glucose and propionate infusions on glucose-entry rates in the lactating and non-lactating cows*
Rates are either hepatic glucose output alone or, when glucose infusion was occurring, hepatic glucose output +4.2. For details of infusion procedures, see the Experimental section. *, $P<0.05$; **, $P<0.01$ (compared with the pre-infusion value for the propionate infusion only).

		Glucose-entry rate (mmol in whole blood/min)					
			Time elapsed during infusion				
Cow status	Treatment	Pre-infusion	4.5h	24h	48h	Post-infusion	Standard error of difference between means
(*a*) Glucose infusion							
Lactating	Glucose-infused	6.52	5.34	6.82	8.44	7.77	—
	Control	5.44	8.71	5.46	6.61	5.46	—
Non-lactating	Glucose-infused	3.58	7.38	5.56	9.03	4.23	—
	Control	3.65	5.66	2.51	3.55	2.48	—
			Time elapsed during infusion				
			0.5h	1.5h	3.0h		
(*b*) Propionate infusion							
Lactating	Propionate-infused	6.74	5.12	6.99	9.22	7.72	1.21
	Control	6.11	6.18	5.81	7.00	7.60	1.49
Non-lactating	Propionate-infused	3.72	8.10**	8.89**	7.26*	4.52	1.21
	Control	2.17	2.09	3.61	2.36	5.47*	1.49

non-lactating cows (Table 1). These values compare with the value of 50% obtained by Brockman & Bergman (1975) in normal fed non-lactating non-pregnant ewes. Uptake by the liver is therefore a major fate for insulin in ruminants as in non-ruminants (see e.g. Mortimore *et al.*, 1959).

Both glucose and propionate have been shown to be potent insulin secretagogues in cattle and sheep (Manns *et al.*, 1967; Trenkle, 1970; McAtee & Trenkle, 1971). The present work extends these observations by demonstrating that, in dairy cows at least, the insulin response to these compounds may depend on lactational status. The low insulin response in the lactating cows in the present study is consistent with the observation of Frobish & Davis (1977) that abomasal infusions of either glucose or propionate for 5-day periods, at 8.3 and 10.4mmol/min respectively, had little effect on circulating insulin concentrations in lactating cows. By contrast, Thompson *et al.* (1975) found an increase in blood-insulin concentration in lactating cows infused intravenously with glucose for 3h at a rate of about 6mmol/min. The difference in these findings might have been due to the difference in the duration of the glucose infusion, since insulin concentration and output were higher in the lactating cows in the present study at 4.5h than at later times during the glucose infusion (Tables 2 and 3). However, the latter phenomenon may have been due to the fact that these cows had recently received their afternoon feed, since corresponding rises in concentration and output are evident in the control animals.

The question arises whether the difference in the insulin secretory response between the lactating and non-lactating cows in the present study was due to a diminished response in the lactating cows or to an exaggerated response in the non-lactating cows. It is difficult to resolve this point owing to the dearth of comparable work with glucose infusions lasting as long as 48h. Comparison with studies with shorter-term infusions, such as that of Lohmann *et al.* (1978), which demonstrated a diminished insulin concentration response to a 2h glucose infusion in highly trained athletes, suggests that it is the response of the lactating cows that is diminished, since the insulin concentrations reached in the athletes and the untrained controls were similar to those reached in the lactating and non-lactating cows respectively in the present work. If this is indeed the case the present study appears to be the first direct demonstration that diminished insulin secretory response can occur in association with lactation *per se*.

It is possible that the differences in insulin response did not relate to lactation at all, but rather were related to the energy balance of the animals (cf. Malaisse *et al.*, 1967; Hedeskov & Capito, 1974). The nutrition of the cows was not strictly monitored in the present study. However, the available data indicated that the lactating cows were in slight negative nutritional balance, perhaps due to the diminished appetite seen in the first 10 weeks of lactation (Technical Bulletin 33, 1975), whereas the non-lactating cows were in positive energy balance (see the Experimental section). Nevertheless, any deviation from strict energy balance that did occur did not appear to affect other metabolic parameters, and comparison of these parameters with previously published values, where available, indicated that the metabolic status of the untreated lactating and non-lactating cows was normal. Thus the pre-infusion values for hepatic glucose output (Table 6) are similar to previously reported values for glucose-entry rates for normal lactating and non-lactating cows (e.g. Leng, 1970). The pre-infusion arterial concentrations of glucose, acetate and hydroxybutyrate in the lactating cows, i.e. 2.74 ± 0.15, 1.48 ± 0.07 and 0.80 ± 0.22 respectively (mM in whole blood; mean $\pm$ S.E.M., $n = 6$ in each case), were all very similar to those found for adequately fed lactating cows by other workers (e.g. Bickerstaffe *et al.*, 1974). The corresponding pre-infusion concentrations in the non-lactating animals were 3.29 ± 0.12, 1.42 ± 0.11 and 0.44 ± 0.02. Apart from these considerations, the glucose infusion was equally well tolerated in both groups of cows (Table 5). By contrast, food-deprived lactating cows show marked glucose intolerance under these circumstances (Treacher *et al.*, 1976). The tolerance to glucose in the present work appeared to be achieved in two different ways, however, Thus, in the non-lactating cows it was achieved by increased insulin secretion and hence, presumably, increased peripheral utilization of glucose. In the lactating cows it was achieved mainly by decrease in hepatic glucose output. Shikama & Ui (1978) have pointed out that these two mechanisms may operate together to achieve glucose tolerance in the starved rat. The latter method, which may involve the mediation of adrenocortical hormones (Shikama & Ui, 1978), may be favoured during lactation, and it is of interest that the blood concentration of cortisol increases with the onset of lactation in dairy cows (Paterson & Linzell, 1974). Significant secretion of insulin in response to a glucose load may only occur in lactating cows if the load is so great that decrease in hepatic glucose output alone will not prevent hyperglycaemia. This may explain the observations of Thompson *et al.* (1975), discussed above, since hyperglycaemia occurred in their study, even though endogenous glucose entry decreased by 68%. In non-lactating cows, decrease in hepatic glucose output may only occur in response to a glucose load when the increase in insulin secretion is insufficient to prevent hyperglycaemia. Finally, it is evident that the intake of metabolizable energy was substantially larger in the lactating cows than in the non-lactating cows (see

the Experimental section). Any relationship between the magnitude of feed intake and insulin response clearly did not therefore apply to the two groups of cows taken together. Robinson *et al.* (1978) have also recently observed that the arterial concentration of insulin is higher in virgin rats than in lactating rats when both groups of animals are fed *ad libitum*.

Other reasons for the difference in insulin response in the lactating and non-lactating cows can be considered. One is that there was a relationship between the blood concentrations of insulin and glucose. During the glucose infusion there was no evident correlation between these two parameters in the two groups of cows taken together. However, there were significant positive correlations between the arterial concentrations of glucose and insulin in both groups of cows considered separately. The regression equations were $y = 10.44x - 13.59$ (correlation coefficient = 0.683; $P<0.01$, and $y = 89.66x - 224.84$ (correlation coefficient = 0.696; $P<0.01$, for the lactating and non-lactating cows respectively, where y is the arterial insulin concentration in m-units/litre, and x the arterial glucose concentration in mM. The values for each equation were obtained from each of the three cows at the five sampling times (i.e. pre-infusion, 4.5h, 24h, 48h and post-infusion). It is evident that the slope of the regression line was much steeper for the non-lactating cows than for the lactating cows. A further consideration is that there could have been a relationship between insulin dynamics and glucose-entry rate. Such a direct relationship is not immediately evident in the untreated animals taken together, since hepatic glucose output was higher in the lactating cows, whereas insulin concentration and portal output were lower. Consideration of the two groups of cows separately, however, showed that in the glucose-infusion experiment there was a significant positive correlation between arterial insulin concentration and glucose-entry rate in the non-lactating cows (Fig. 1; cf. Bassett *et al.*, 1971), but not in the lactating cows. Lastly, it may be suggested that, during lactation, there is some direct effect on the pancreas that results in a decrease in the ability of this organ to secrete insulin. Such an effect could perhaps be mediated by a hormone involved in regulating lactation. That sex hormones can influence the insulinogenic activity of the pancreas is demonstrated by the fact that progesterone increases the ability of the β-cells to secrete insulin (Ashby *et al.*, 1978).

Recent research has suggested that insulin may play only a minor role in regulating glucose transport into the mammary gland of the rat (Robinson & Williamson, 1977). If this really is the case, and if a similar situation pertains in ruminants, then a low circulating concentration of insulin could be of value to the dairy cow during lactation in directing available glucose to the mammary gland, and thereby helping

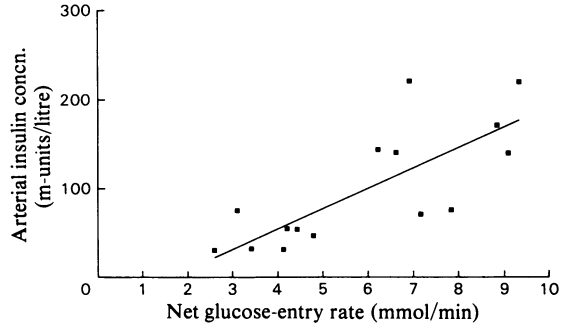

Fig. 1. *Relationship between glucose-entry rate and arterial insulin concentration in the non-lactating cows in the glucose-infusion experiment*

The values are those obtained for each of the three cows at the five sampling times (i.e. pre-infusion, 4.5h, 24h, 48h and post-infusion). The equation of the line is $y = 22.67x - 32.48$ and the correlation coefficient = 0.775 ($P<0.001$).

to maintain milk output, if the well-established insulin sensitivity of competing tissues such as skeletal muscle and adipose tissue (cf. Randle *et al.*, 1964; Yang & Baldwin, 1973) is unaltered. It remains to be seen whether the diminished insulin secretion of lactating cows is an adaptation to glucose shortage in ruminants, or whether it is a feature of lactation in non-ruminant species as well (Robinson *et al.*, 1978). A propensity to low insulin concentrations in high-milk-yielding cows could be a precipitating cause of bovine ketosis (cf. Schwalm & Schultz, 1976; Hove & Halse, 1978), since a decline in the availability of glucose and acetate (Jarrett *et al.*, 1972) to peripheral tissues, other than the mammary gland, would be accompanied by increased mobilization of non-esterified fatty acids and increased hepatic ketogenesis.

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