Non-insulin- and insulin-mediated glucose uptake in dairy cows

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Summary. Four mid-lactation Holstein dairy cows (mean milk yield on day of experiments 26·1 kg/d) were used in a series of experiments to establish the contribution of non-insulin-mediated glucose uptake to total glucose uptake at basal insulin concentrations. A secondary objective was to determine whether somatostatin affects the action of infused insulin. In part I of the experiment a primed continuous infusion of [6,6-^{2}H]glucose (45·2 μg/kg per min) was begun at time 0 and continued for 5 h. After 3 h of [6,6-^{2}H]glucose infusion (basal period) a primed continuous infusion of insulin (0·001 i.u./kg per min) was administered for 2 h. Coincident with the insulin infusion, normal glucose was also infused in order to maintain the plasma glucose concentration at euglycaemia. Part II of the experiment was the same as part I except that somatostatin was infused for 2 h (0·333 μg/kg per min) instead of insulin. In part III of the experiment both insulin and somatostatin were infused for the final 2 h. Plasma insulin levels were increased by insulin infusion (to 0·1476 and 0·1290 i.u./l for parts I and III respectively) and were reduced by somatostatin infusion in part II (to 0·006 i.u./l) relative to the basal periods (mean 0·021 i.u./l). Glucose uptake during somatostatin infusion (2·50 mg/kg per min; part II) was 92·0 % of that observed in the respective basal period (2·72 mg/kg per min). Circulating insulin levels were much lower than the dose of insulin that causes a half maximal effect on glucose uptake (0·06–0·10 i.u./l for ruminants); consequently insulin-mediated glucose uptake was probably absent in part II. Secondly, glucose uptake following insulin only infusion (4·05 mg/kg per min) was significantly lower than that observed when insulin plus somatostatin was infused (4·69 mg/kg per min), indicating that somatostatin either directly or indirectly enhanced the action of insulin on glucose uptake.

Glucose uptake by tissues is controlled by both insulin-dependent and insulin-independent pathways, in ruminants and non-ruminants. However, although numerous studies have examined the relationship between insulin and glucose utilization in ruminants (Weekes *et al.* 1983; Sano *et al.* 1993) relatively few have determined non-insulin-mediated glucose (NIMG) uptake as a proportion of total glucose utilization. Using regression analysis, Janes *et al.* (1985) extrapolated glucose turnover to a zero insulin concentration from normal and elevated plasma insulin concentrations in sheep, and determined that NIMG uptake is ~ 65 % of total

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glucose turnover. However, this method of calculation makes the assumption that the increase in glucose turnover with increasing insulin concentration is linear at low plasma insulin concentrations, an assumption not necessarily true. As far as we are aware there have been no attempts to determine NIMG uptake in lactating dairy cows. However, as the ruminant mammary gland is insensitive to insulin (Laarveld *et al.* 1981) but accounts for ~50–80% of whole body glucose turnover at peak lactation (Annison, 1983) it is likely that NIMG uptake will represent an even greater proportion of total glucose turnover. In studies in humans and other non-ruminants, somatostatin has been infused which prevents insulin release from the pancreas, reducing plasma insulin to below detectable levels (Baron *et al.* 1987*b*). Under such conditions measurements of glucose turnover should be entirely non-insulin-mediated. Using this technique it has been established that insulin-mediated glucose (IMG) uptake represents ~40% of total post-absorptive glucose turnover in humans, the remaining 60% being NIMG uptake (Baron *et al.* 1987*a*, *b*). Thus, the primary aim of the present experiment was to determine the level of NIMG uptake in lactating dairy cows by preventing insulin release by infusing somatostatin.

In dogs, the concomitant infusion of somatostatin and insulin has been shown to increase glucose turnover to a level greater than that observed by infusing insulin alone (Bergman *et al.* 1984). However, another group using humans have failed to show any such enhanced response to insulin with somatostatin infusion (Baron *et al.* 1987*b*). Thus, a secondary objective of the present work was to establish whether IMG turnover in dairy cows is enhanced by the infusion of somatostatin.

Materials and methods

Animals

A total of 12 experiments were performed on four lactating Holstein dairy cows. The animals were 205 ± 13 d post partum (values are means ± SE) and had an average milk yield of 26·1 ± 2·2 kg/d on the day of the experiments. The weight of the cows was 587·2 ± 16·0 kg. The animals were housed in stanchions with constant access to water and were allowed to exercise daily between 16.00 and 18.00. The cows were milked daily at 09.00 and 18.00 and the milk yields recorded. Morning and evening milk samples were retained once each week for the determination of milk components. The animals were fed so as to maintain body weight and milk yield, using the Japanese Feeding Standard (Anon. 1987). Hay comprised 40% of the diet and a commercial compound concentrate 60%. The hay portion of the diet was given at 10.00 and 18.00 and the concentrate portion in 12 equal amounts using an automatic feeder at 01.00, 03.00, 05.00, 07.00, 10.00, 12.00, 13.30, 15.30, 19.00, 20.30, 22.00 and 23.30. The cows were allowed to adjust to these conditions for at least 21 d before any experiments were performed.

Experimental procedure

A minimum of 1 week elapsed between experiments on the same animal in order for the stable isotope of glucose (used for turnover measurements) to clear from the circulation. The experiments were performed in a randomized block design. At 24 h prior to an experiment the jugular veins on both sides of the neck were catheterized to permit the infusions and blood sampling. In part I of the experiment from time 0, a primed (3·7 mg/kg) continuous infusion of [6,6-^{2}H]glucose (45·2 μg/kg per min at 0·65 ml/min) was begun and continued for 5 h. Immediately before the isotope infusion, a blood sample was taken in order to determine the background isotope level. After 180 min, a primed infusion of insulin (0·001 i.u./kg per min at 1·5 ml/min;

Actrapid monocomponent porcine insulin; Novo Industri, DK-2880 Bagsværd, Denmark) began and continued for 120 min (Rose *et al.* 1996*b*). Coincident with the insulin infusion normal glucose was infused in order to maintain the plasma glucose concentration at the level noted in the basal period. The plasma concentration of glucose was rapidly determined from 1 ml blood samples taken every 10 min, using a YSI-Glucose Analyzer (Yellow Springs Instruments, Yellow Springs, OH 45387, USA) and this was used to adjust the normal glucose infusion rate. For the last 40 min of the basal period and the insulin infusion period the animals were assumed to have reached a steady state with respect to glucose turnover and all measurements were taken during these so-called 'plateau periods'. During the plateau periods large (15 ml) blood samples were taken into heparinized test tubes every 10 min; additional large blood samples were taken at 20 min intervals between the plateau periods. The blood samples were stored on ice until they were centrifuged and the plasma stored at −20 °C immediately after the experiment. The design of part II of the experiment was the same as that of part I except that somatostatin was infused for 2 h (0·333 μg/kg per min at 0·65 ml/min) from time 180 min instead of insulin. Glucose was also infused in order to maintain the plasma glucose concentration constant. In part III of the experiment both insulin and somatostatin were infused at the same rates as for parts I and II. Infusion solutions were sterilized using sterile 0·2 μm polysulphone filters (Kanto Chemical Co. Inc., Tokyo 103, Japan).

In part I of the experiment only, as soon as insulin had been infused for 2 h, the [6,6-^{2}H]glucose infusion was stopped, but the insulin and variable glucose infusions continued so that the plasma glucose concentration was kept at a constant level. Blood samples were taken at 5, 10, 20, 30, 45 and 60 min after the end of the [6,6-^{2}H]glucose infusion in order to calculate the cows' rapidly mixable glucose pool size from the 'washout' of isotope.

Analytical methods

The plasma concentrations of insulin and growth hormone were determined using radioimmunoassay procedures as described by Fuller *et al.* (1977) and Johke *et al.* (1978) respectively. Plasma concentrations of non-esterified fatty acids were determined using a commercially available kit (Wako Chemicals Co. Ltd, Osaka 541, Japan). Plasma α-amino nitrogen concentrations were determined according to Ibbot (1974). Samples for the measurement of the enrichment of [6,6-^{2}H]glucose were prepared using a method modified from Wiecko & Sherman (1976) as described by Rose *et al.* (1996*b*). Milk concentrations of fat, protein and lactose were determined using a Milkoscan 203B milk analyser (Foss Electric, DK-3400 Hillerød, Denmark).

Calculations and statistics

The rate of glucose infused to maintain euglycaemia during the insulin and somatostatin infusions was calculated at 10 min intervals and expressed as mg/kg per min. The glucose infusion rate represents the sum of the effects of the insulin and somatostatin on glucose utilization in peripheral tissues and the suppression of gluconeogenesis at the liver, and was thus a measure of the overall effect of insulin and somatostatin on glucose metabolism.

The rapidly mixable pool of glucose was determined by fitting a double exponential equation to the 'washout' of [6,6-^{2}H]glucose (Katz *et al.* 1981). The non-steady state equations of Steele *et al.* (1956) as modified by Cowan & Hetenyi (1971) were applied to the results to assess the rate of glucose appearance (*Ra*) and rate of glucose disappearance (*Rd*). The sliding fit of triplicates technique was used so that

non-steady state measurements represented average changes over three consecutive measurements of plasma glucose concentration and molar percent excess (Cherrington & Vranic, 1973; Argoud *et al.* 1987). The rate of endogenous glucose output was determined by subtracting the glucose infusion rate (required to maintain a constant plasma glucose concentration) from *Ra*. The plasma glucose metabolic clearance rate (MCR) was also determined by dividing *Rd* by the plasma glucose concentration at a specific time.

All the results presented are average values for the plateau periods. Differences in measures of glucose turnover and the plasma concentrations of hormones and metabolites between the three parts of the experiment, as well as between the basal and insulin and/or somatostatin infusion periods, were determined by paired Student's *t* tests.

RESULTS

Relatively constant plasma glucose concentrations were maintained during the plateau periods of the present experiment. The average CV during the plateau periods was $4{\cdot}80 \pm 0{\cdot}56\,\%$ and in no case was it $> 9\,\%$. The size of the rapidly mixable glucose pool, calculated from the 'washout' of labelled glucose following the end of the part I experiments, was $121{\cdot}5 \pm 4{\cdot}66$ ml/kg body weight. Values are given throughout as means $\pm$ SE.

The average plateau plasma glucose concentrations were not significantly different either between the stages of the experiment or between the basal and infusion periods (Table 1). The glucose infusion rate required to maintain a constant plasma glucose concentration during the insulin only infusion was significantly lower than that required when both insulin and somatostatin were infused (Table 1). The difference was $\sim 20\,\%$. The amount of glucose required when insulin was infused (parts I and III) was significantly greater than that required when just somatostatin was infused. During the somatostatin only infusions, the normal glucose infusion rate increased to $\sim 0{\cdot}8$ mg/kg per min within ~ 30 min of the peptide being infused. Thereafter, the amount of normal glucose required decreased to 0, usually within ~ 80 min of the somatostatin infusion beginning. Following this, plasma glucose concentrations remained stable in the absence of exogenous glucose infusion.

The values for *Ra* and *Rd* were very similar at each stage of the experiment, indicating that good steady state conditions were maintained with respect to glucose turnover. During the basal (control) periods, neither *Ra* nor *Rd* differed significantly between parts I and II of the experiment (Table 1). However, in the basal period of part III both *Ra* and *Rd* were higher than their respective values in part II. Following the infusion of insulin (parts I and III), *Ra* increased significantly. Infusion of somatostatin only had no significant effect on either *Ra* or *Rd*. During the infusion of insulin only, *Ra* and *Rd* were significantly lower than the respective values for the infusion of insulin and somatostatin together. During the somatostatin only infusion, *Rd* represented NIMG uptake, which in the present experiment was $2{\cdot}499$ mg/min per kg. This was $92{\cdot}0 \pm 2{\cdot}94\,\%$ of the *Rd* value noted in the basal period.

The glucose MCR was not significantly different between the different stages of the experiment in the basal periods (Table 1). Glucose MCR over the course of the experiment is shown in Fig. 1. Following an initial small dip immediately after the start of the insulin infusions MCR increased rapidly, the rate of increase being significantly greater for the insulin and somatostatin infusion than when insulin was infused alone. Relatively stable conditions were maintained in the final 40 min of the

Table 1. *Plasma glucose concentration and characteristics of glucose kinetics during insulin (part I), somatostatin (part II) or insulin and somatostatin (part III) infusions in dairy cows*

		Stage of experiment			Between stage of experiment†		
		Part I	Part II	Part III	I *v.* II	I *v.* III	II *v.* III
Plasma glucose, mg/l	Basal	665·5	657·5	661·5	NS (10·23)	NS (16·59)	NS (19·40)
	Infusion	662·0	654·0	636·0	NS (15·81)	NS (18·20)	NS (16·21)
	Basal *v.* infusion‡	NS (17·55)	NS (13·10)	NS (12·92)			
Glucose infusion rate, mg/kg per min	Infusion	2·338	0·180	2·995	** (0·205)	* (0·180)	*** (0·163)
Rate of appearance, mg/kg per min	Basal	2·679	2·711	2·953	NS (0·160)	NS (0·119)	* (0·045)
	Infusion	4·097	2·518	4·741	*** (0·243)	* (0·143)	*** (0·143)
	Basal *v.* infusion‡	*** (0·105)	NS (0·116)	** (0·182)			
Rate of disappearance, mg/kg per min	Basal	2·684	2·715	2·887	NS (0·159)	NS (0·125)	* (0·041)
	Infusion	4·050	2·499	4·688	* (0·292)	* (0·196)	** (0·219)
	Basal *v.* infusion‡	** (0·111)	NS (0·070)	** (0·228)			
Metabolic clearance rate, ml/kg per min	Basal	4·017	4·101	4·355	NS (0·296)	NS (0·172)	NS (0·195)
	Infusion	6·223	3·805	7·495	* (0·597)	* (0·329)	** (0·358)
	Basal *v.* infusion‡	** (0·315)	* (0·089)	*** (0·162)			
Endogenous glucose output, mg/kg per min	Infusion	1·759	2·341	1·742	NS (0·228)	NS (0·060)	NS (0·285)

† Significance of difference between respective stages of experiment with SED in parentheses.
‡ Significance of difference between basal and infusion plateau periods with SED in parentheses.
NS, no significant difference; *, $P < 0·05$; **, $P < 0·01$; ***, $P < 0·001$.

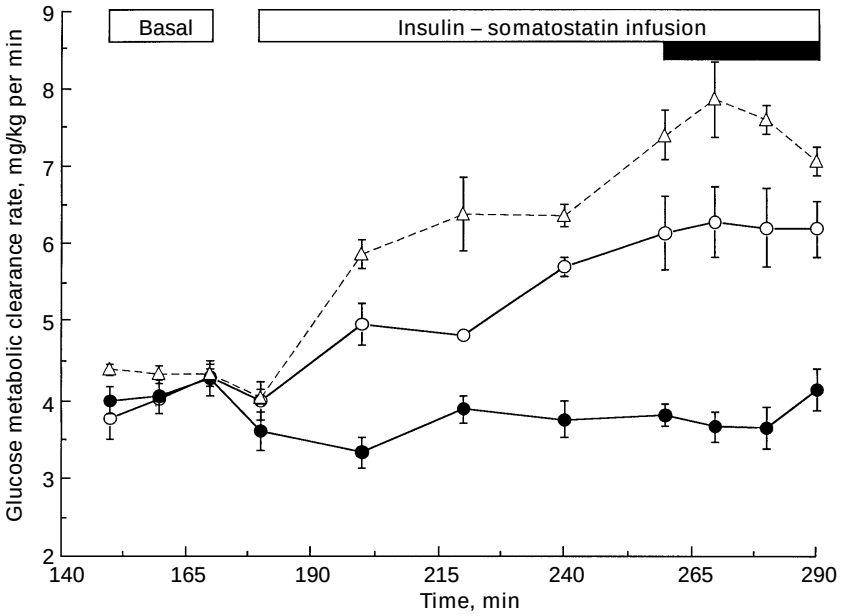

Fig. 1. Glucose metabolic clearance rate in dairy cows during the basal plateau period and during the infusion of ○, insulin; ●, somatostatin or △, insulin plus somatostatin. Values are means for $n = 4$ with SEM indicated by vertical bars. ▬, Infusion plateau period.

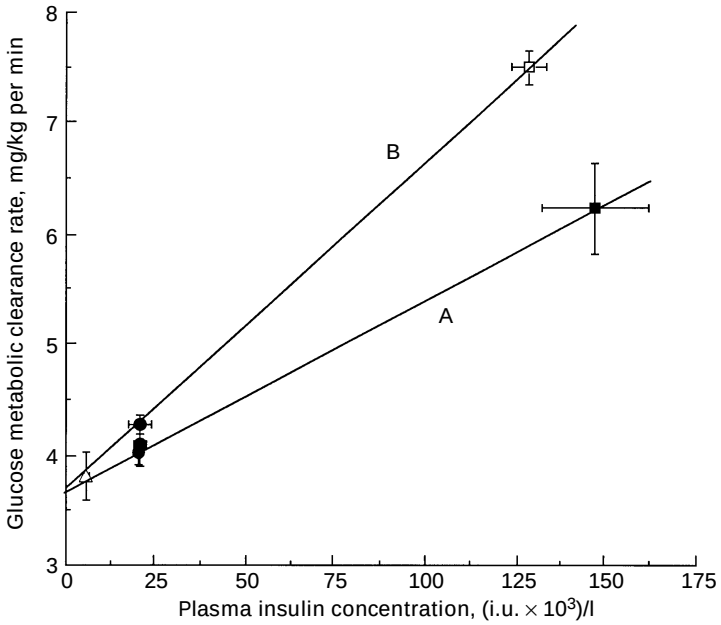

Fig. 2. Relationships between plasma insulin concentration and glucose metabolic clearance rate in dairy cows during ●, the basal period and infusion of △, somatostatin; ■, insulin or □, insulin plus somatostatin. Values are means for $n = 4$ with SEM indicated by vertical bars. Regression equation A (utilizing the basal and insulin only values): $y = 31x + 3{\cdot}520$ ($r^2 = 0{\cdot}982$). Regression equation B (utilizing the basal and insulin plus somatostatin values): $y = 16x + 3{\cdot}821$ ($r^2 = 0{\cdot}993$).

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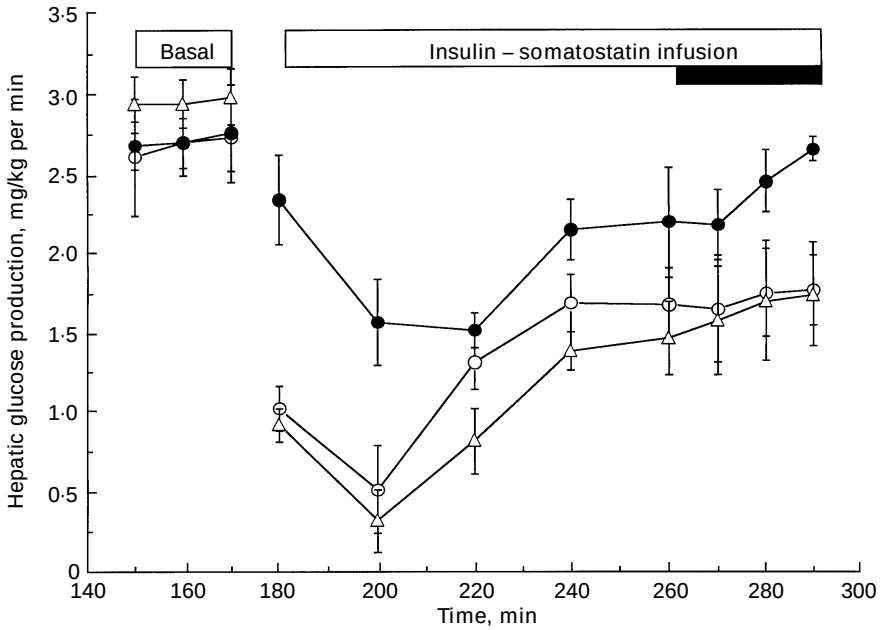

Fig. 3. Endogenous glucose production in dairy cows during the basal plateau period and during the infusion of ○, insulin; ●, somatostatin or △, insulin plus somatostatin. Values are means for $n = 4$ with SEM indicated by vertical bars. ▬, Infusion plateau period.

experiments. When somatostatin was infused alone MCR decreased by ~ 20% and then slightly increased, but was still significantly lower at the end of the experiment than in the basal period. The MCR noted during part II of the experiment represented NIMG clearance, which was 3·805 mg/min per kg. This was 92·8 ± 2·28% of the MCR value noted in the basal period. Consequently, we estimated that at basal (i.e. normal) plasma insulin concentrations, in the cows in the present experiment IMG turnover represented 7·2% of total glucose clearance. The value for NIMG clearance (3·805 mg/min per kg) from part II of the experiment was used to calculate IMG clearance when plasma insulin concentrations were increased to 0·130–0·150 i.u./l. The values obtained were 38·3 ± 3·75% of total glucose clearance during the insulin only infusion and 49·0 ± 3·87% when insulin and somatostatin were infused together. These latter two values, which were significantly different ($P < 0·05$, SED 3·37), were calculated assuming that all the extra glucose infused was utilized by insulin-mediated pathways. The relationship between glucose MCR and plasma insulin concentration in the different parts of the present experiment is shown in Fig. 2. When the linear regressions of Fig. 2 were used to extrapolate to a zero insulin concentration, then NIMG clearance as a proportion of the respective glucose clearances at basal insulin concentrations became 91·07 ± 1·39 and 86·44 ± 2·23% for parts I and III of the experiment respectively. These values were not significantly different ($P > 0·1$, SED 2·099); determined by extrapolation, they were also not significantly different from the NIMG clearance determined from part II of the experiment ($P > 0·1$, SED 1·328 and $P > 0·05$, SED 2·527 for the values determined from parts I and III respectively).

The rate of endogenous glucose output during the experiments is shown in Fig. 3. At the start of the infusions, glucose output greatly decreased (relative to the *Ra* observed during the basal period) and subsequently increased, but was still

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Table 2. *Plasma concentrations of hormones and non-esterified fatty acids during insulin (part I), somatostatin (part II) or insulin and somatostatin (part III) infusions in dairy cows*

		Stage of experiment			Between stage of experiment†		
		Part I	Part II	Part III	I *v.* II	I *v.* III	II *v.* III
Insulin, i.u./l	Basal	0·020	0·021	0·021	NS (0·0019)	NS (0·0021)	NS (0·0024)
	Infusion	0·148	0·006	0·129	** (0·0155)	NS (0·0100)	*** (0·0057)
	Basal *v.* infusion‡	** (0·0144)	** (0·0017)	*** (0·0046)			
Glucagon, μg/l	Basal	102·8	110·9	106·0	NS (3·92)	NS (6·05)	NS (5·96)
	Infusion	94·5	75·0	69·1	NS (7·06)	* (7·17)	* (1·81)
	Basal *v.* infusion	NS (5·48)	*** (2·55)	* (7·08)			
Growth hormone, μg/l	Basal	3·39	2·80	2·91	NS (0·43)	NS (0·31)	NS (0·521)
	Infusion	1·50	0·92	0·91	NS (0·28)	NS (0·30)	NS (0·103)
	Basal *v.* infusion	NS (0·86)	NS (0·92)	NS (0·81)			
Cortisol, μg/l	Basal	0·70	0·73	0·50	NS (0·075)	* (0·061)	* (0·053)
	Infusion	0·96	2·47	0·62	NS (2·11)	NS (0·21)	NS (1·91)
	Basal *v.* infusion	NS (0·098)	NS (1·97)	NS (0·12)			
Non-esterified fatty acids, μequiv./l	Basal	94·9	88·6	92·5	NS (10·56)	NS (5·25)	NS (8·70)
	Infusion	61·0	186·0	42·0	NS (116·8)	** (2·55)	NS (116·2)
	Basal *v.* infusion	* (8·38)	NS (119·28)	** (4·63)			

† Significance of difference between respective stages of experiment with SED in parentheses.
‡ Significance of difference between basal and infusion plateau periods with SED in parentheses.
NS, no significant difference; *, $P < 0{\cdot}05$; **, $P < 0{\cdot}01$; ***, $P < 0{\cdot}001$.

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$\sim$ 35–40 % lower (in the case of parts I and III) and $\sim$ 15 % lower (in the case of part II) at the end of the experiment than the *Ra* observed in the basal period. There were no differences between the infusions in the rate of hepatic glucose production in the plateau periods at the end of the experiment.

The plasma concentration of insulin was greatly increased by the insulin infusions (parts I and III), though there was no significant difference in insulin concentration between the insulin and insulin plus somatostatin infusions (Table 2). Following the infusion of somatostatin there was a significant reduction in the plasma concentration of insulin, until relatively stable levels ($\sim$ 0·006 i.u./l) were observed. The plasma concentrations of glucagon were also significantly lower following the infusion of somatostatin (in parts II and III), though no effect was observed when insulin alone was infused (Table 2). The plasma concentration of growth hormone was not significantly affected by the insulin or somatostatin infusions, though the values were numerically reduced during the infusions when compared with the values in the basal periods (Table 2). The plasma concentrations of growth hormone were not significantly different between the different infusion experiments, in either the basal or infusion plateau periods. The plasma concentration of cortisol was not significantly affected by any of the infusions in the present experiment, though the value in the basal period of part III was significantly lower than that in the other experiments (Table 2). The plasma concentration of non-esterified fatty acids was significantly reduced by the somatostatin plus insulin and insulin alone infusions, though there was no significant effect of somatostatin alone (Table 2). The plateau non-esterified fatty acids value in part III of the experiment was significantly lower than that found in part I.

The mean concentrations of fat, protein and lactose in the milk samples over the 3 weeks of experimentation were 39·3 $\pm$ 3·8, 32·7 $\pm$ 1·3 and 42·9 $\pm$ 1·3 g/kg respectively.

DISCUSSION

The present experiment has demonstrated that the proportion of basal glucose uptake that is mediated by insulin in fed mid-lactation dairy cows is relatively small, at $\sim$ 8 % of basal glucose utilization. This value compares with 18 % for fed non-pregnant, non-lactating sheep in the study of Janes *et al.* (1985), in which glucose uptake was extrapolated to a zero plasma insulin concentration from basal and elevated insulin levels. Rates of IMG uptake as a proportion of total glucose utilization in normal humans, determined using methods similar to those in the present study, as well as methods using linear regression analysis, at postprandial basal insulin levels have been in the range 15–30 % (Gottesman *et al.* 1983; Baron *et al.* 1988). In the present experiment, IMG uptake increased to 38·5 % when insulin concentrations were increased to $\sim$ 6-fold those of basal levels.

In the present experiment, the values of NIMG clearance obtained following severe hypoinsulinaemia induced by somatostatin and by extrapolation from elevated insulin concentrations were largely similar, indicating a similar applicability for both techniques. Estimates of NIMG clearance achieved following extrapolation from values of glucose clearance obtained from concomitant infusion of insulin and somatostatin are, however, possibly less reliable, even if in the present experiment the value from part III was not quite statistically significantly different from the value obtained by insulin only infusions. Ideally, a number of insulin infusion rates should have been used in the present experiment to ensure that glucose clearance varied linearly with insulin concentration. However, several studies have found that

this relationship is linear over this range of plasma insulin concentrations in cows and sheep (Janes *et al.* 1985; Rose & Obara, 1996; Rose *et al.* 1996*b*).

The mammary gland has been shown to account for some 50–80% of the total glucose entry rate into the body in lactating cows, depending upon milk yield (Annison, 1983), and as it is widely accepted that the ruminant mammary gland is insensitive to insulin (Laarveld *et al.* 1981) milk production probably accounts for a very large proportion of NIMG uptake in lactating animals. Indeed, it has been calculated that 1 l of milk requires a mammary glucose uptake of ~ 72 g (Kronfeld, 1982). If this figure is applied to the milk yield observed on the experimental days of the present study then it can be calculated that ~ 88% of total NIMG uptake (or ~ 82% of total glucose turnover at normal insulin concentrations) can be accounted for by the mammary gland.

In the basal periods, glucose turnover was greater in part III than in part II only when expressed as *Ra* and *Rd*, but not when expressed as MCR. MCR is the preferred measure of glucose turnover when there are small variations in the plasma glucose concentration because expressing turnover rate as MCR removes the effect that plasma glucose concentration has on the rate of its own disposal, provided changes in glucose concentration are relatively modest.

There was a very clear effect in the present experiment for the somatostatin infusion to increase the turnover of glucose during concomitant insulin infusion. It has previously been noted that when insulin and somatostatin (at 48·2 μg/kg per h) were infused into dogs, there was a similar, though more striking (65%), increase in the clearance of glucose from plasma than when insulin alone was infused at the same rate (Bergman *et al.* 1984). However, conflicting results have been obtained from another study in humans, in which somatostatin had no effect on glucose clearance during insulin infusion (Baron *et al.* 1987*b*). The latter authors suggested that the conflicting results may be due to the dose of somatostatin used; Baron *et al.* (1987*b*) used a lower rate of somatostatin infusion than either the present study or that of Bergman *et al.* (1984). The mechanism by which somatostatin increases insulin action is unclear. The largely similar rates of endogenous glucose output in the plateau periods of parts I and III indicate that the effect of the somatostatin was peripheral rather than on the liver. Somatostatin also failed to evoke an increase in glucose turnover when infused alone into the arms of humans (Capaldo *et al.* 1986), and thus the result in the present experiment was unlikely to be due to somatostatin directly causing an increase in glucose uptake. Consequently, somatostatin possibly either directly increased the sensitivity of the tissues to insulin, or some secondary effect of somatostatin subsequently increased insulin sensitivity. A direct effect of somatostatin on insulin action would require that there were somatostatin receptors on insulin-sensitive tissues. While we are not aware of any reports demonstrating the existence of somatostatin receptors on insulin-sensitive tissues in the ruminant, somatostatin receptors have been demonstrated *in vitro* in adipose tissue and cardiac muscle in the rat (Simon *et al.* 1988; Patel *et al.* 1995). It is possible that a reduction in growth hormone concentration may mediate the effect of somatostatin. However, the non-significant, short-term growth hormone reduction noted in the present experiment is unlikely to have such a large and immediate effect on insulin action; certainly no such effect has been noted for humans or dogs (MacGoreman *et al.* 1981; Bergman *et al.* 1984). Additionally, a short-term reduction in glucagon levels is also unlikely to have a large effect on peripheral glucose utilization in ruminants (Brockman, 1986). It is not possible from the present experiments to determine what mechanism is responsible for the effect noted.

The plasma concentration of insulin was not completely suppressed by the somatostatin infusion, averaging 0·006 i.u./l during the part II plateau period. In the report of Rose *et al.* (1996*a*) it was shown that complete suppression of immunoreactive insulin concentrations was not possible in sheep using somatostatin, the minimum concentration achieved being ~ 0·005 i.u./l. To measure rates of NIMG uptake it is necessary to induce a severe condition of insulin deficiency. In the present experiment plasma insulin concentrations fell immediately following the somatostatin infusion and remained close to or below the minimum detectable dose for the length of the study; it is therefore possible that there was a low level of IMG uptake. However, given that the sensitivity (i.e. the dose causing half maximal response) of sheep to insulin is between 0·06 and 0·10 i.u./l (Weekes *et al.* 1983; Janes *et al.* 1985) such very low circulating levels are unlikely to have caused a measurable effect on glucose utilization. It is also possible that other blood components or artifacts cross reacted with the insulin antibody used in the assay, apparently increasing the level of immunoreactive insulin present.

Following the onset of the somatostatin and insulin infusions there was a decrease in the rate of hepatic glucose production in all three parts of the experiment. In the case of somatostatin infusion alone (part II) an initial decline in glucose production exceeded utilization. The natural result of such an imbalance is hypoglycaemia which was prevented by the exogenous infusion of glucose. This sequence of events may be the result of a faster onset of the deactivation of the effect of glucagon on the liver relative to that of insulin (Gray *et al.* 1982), leading to the unopposed inhibitory action of insulin on hepatic glucose production (Lavelle-Jones *et al.* 1987). Consequently, the decline in hepatic glucose output would leave insufficient glucose for requirements in the periphery. Later, insulin would also clear from the circulation, removing the inhibition on gluconeogenesis, therefore reducing the external glucose infusion rate required for euglycaemia to zero. The infusion of insulin (parts I and III) caused the well established partial inhibitory effect of insulin on hepatic glucose production (Weekes *et al.* 1983), though the concomitant infusion of somatostatin apparently had no additional significant effect on hepatic glucose output.

The present study has demonstrated that IMG clearance in dairy cows is relatively small at ~ 7·2%. The small value, relative to values for other species, is due to the large NIMG requirement by the mammary gland. When plasma insulin levels were increased to ~ 0·150 i.u./l, IMG clearance increased to 38·3% of total glucose turnover. Additionally, somatostatin apparently either directly or indirectly increased the action of insulin on whole body glucose turnover, possibly by making tissues more sensitive to insulin.

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