CHAPTER 5. RESULTS AND DISCUSSION

1. Introduction

Homeorhetic mechanisms establish insulin resistance during lactation, to spare glucose for the synthesis of the large amount of lactose, lost from the body in milk (Bell & Bauman, 1997). This was accompanied by a loss of body reserves and a reduction in body weight, seen here as a loss of 53.5 kg, or 8.7% of the starting body weight. The loss of body adipose tissue reserve was especially pronounced, as peripheral tissues depend greatly on the fat reserves to supply in their energy requirements and glycerol contributed as hepatic substrate for the additional synthesis of glucose (Bauman & Currie, 1980). The average reduction in BCS experienced by cows in the experiment was 0.3 units, which related to a reduction of 9.9%.

1.1 Production data

Although no statistical analyses were performed on the production data collected in the basal or experimental period, some general remarks can be made, especially in comparison to a group of cows from the herd, not used in the experiment (Figure 47). The decrease in body weight of 31.3 kg (– 5.1%) over the first 8 weeks of the experiment when cows were producing an average of 28.2 kg/d was similar to a decrease in body weight of 52 kg observed in Holstein cows producing between 32.0 to 39.2 kg/d (Andersen et al., 2004). While cows in this experiment lost 0.24 units of BCS (–7.6%), a loss of 1.0 unit was reported between weeks 0 to 8 postpartum in cows by Andersen et al., 2004. The apparent loss of body weight continued into the experimental period and by week 12 postpartum the loss of body weight was 53.5 kg (–8.7%) and the loss of condition reached 0.31 units (–9.9%). This general trend for a decrease in body weight between weeks 8 to 12 postpartum seemed to be a result of treatments, as other cows in the herd experienced an increase in body weight to reach +2.1% of the body weight of week 1 by 12 weeks postpartum. However, the average milk production over the first 8 weeks of the experiment was only 24.2 kg/d in the cows in the herd compared to 28.2 kg/d in experimental cows (+16.4%).
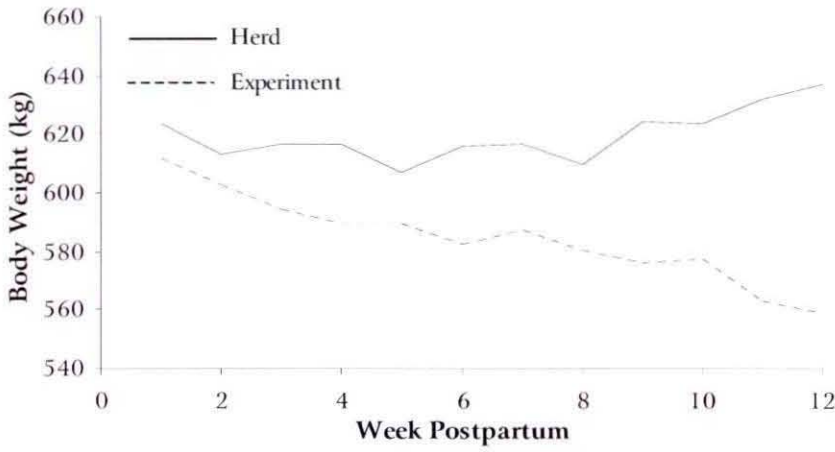
Figure 47. Body weight change in the herd

The decline in BCS between weeks 8 to 12 postpartum similarly seemed to be a result of treatments, rather than a normal trend as lactation progressed (Figure 48). The milk production of the experimental cows seemed greater than the other cows of the herd (Figure 49), with no immediately apparent effect of recombinant bST and a decline in production (lack of maintenance of production) during nutrient restriction.
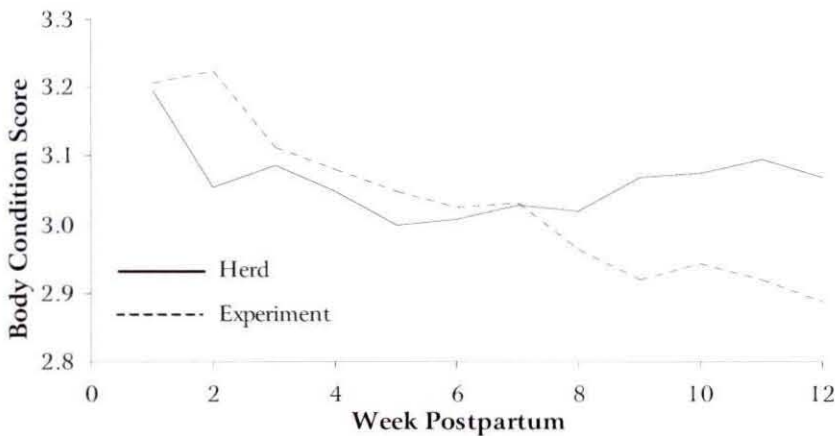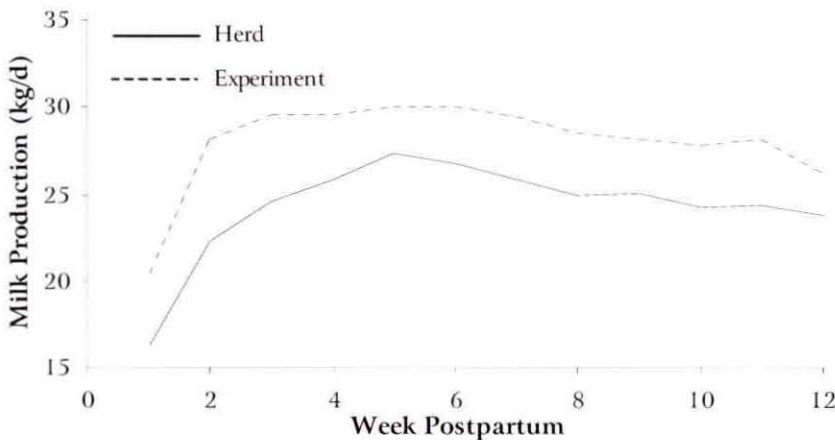

Figure 48. BCS change in the herd

Figure 49. Milk production in the herd

1.2 Average glucose concentration

The plasma glucose concentrations observed in the experiment are in agreement with values reported previously for early lactation dairy cows. These values ranged between 56.0 mg/dL in cows producing 28.3 kg/d milk in week 12 postpartum (Peel *et al.*, 1982) and 76.6 mg/dL at week 5 in cows producing 26.8 kg/d at peak (McDowell *et al.*, 1987b). Cows excluded from the experiment, suspected of being ketotic, had 33.7% lower whole-blood glucose concentration (32.3 mg/dL) compared to experimental cows (48.7 mg/dL) coupled to extremely poor responses of glucose to insulin injection (Figure 8 in Materials and Methods). These results were remarkably similar to the results of experiments using ketotic early lactation dairy cows, where serum glucose concentrations of ketotic cows were 38.0 mg/dL compared to 50.1 mg/dL in non-ketotic cows (Sakai *et al.*, 1996). This was an apparent decrease of 24.2% although these values were not statistically compared and poor glucose clearance during ketosis was also suggested (Sakai *et al.*, 1996).

Normally the plasma glucose concentration decreased from parturition to reach its lowest levels at approximately week 2 postpartum, from where the concentration increased slightly toward week 8 postpartum (Busato *et al.*, 2002, Andersen *et al.*, 2004). The trend toward a rise in glucose concentration was also observed in the cows in the herd where the plasma glucose concentration of week 11 postpartum (65.0 mg/dL) was 7.0% greater than concentrations at week 1 postpartum (60.7 mg/dL). A similar overall trend was apparent in the experimental cows where plasma glucose increased by 11.6%, from 57.6 mg/dL in week 1 to 54.3 mg/dL in week 12 postpartum. There was an uncharacteristically high baseline plasma glucose concentration in samples collected during the control period in week 8 postpartum (Figure 50). This unexpected peak in plasma glucose concentration (+16.4% of week 1) could be ascribed to a stress response of cows exposed to the conditions of the experimental protocol for the first time. However, the second baseline yielded similarly elevated glucose concentrations (65.0 mg/dL) that were stable following the insulin challenge. Sympathetic discharge does not seem a reasonable explanation for this continued hyperglycaemia and cows were given more time to get used to the conditions of the experimental protocols during the control period than any other period. A confounding effect of the analyses of plasma glucose samples can also be eliminated because the glucose concentration of whole-blood was similarly elevated in the control

period compared to the other experimental periods and assays were evenly distributed over the duration of the experiment. The cause of the apparent hyperglycaemia in week 8 (the control period) remains unresolved and was a potentially compromising effect on the results and comparisons made to this period, as the cause of hyperglycaemia could also have confounded the results of the metabolic tests.
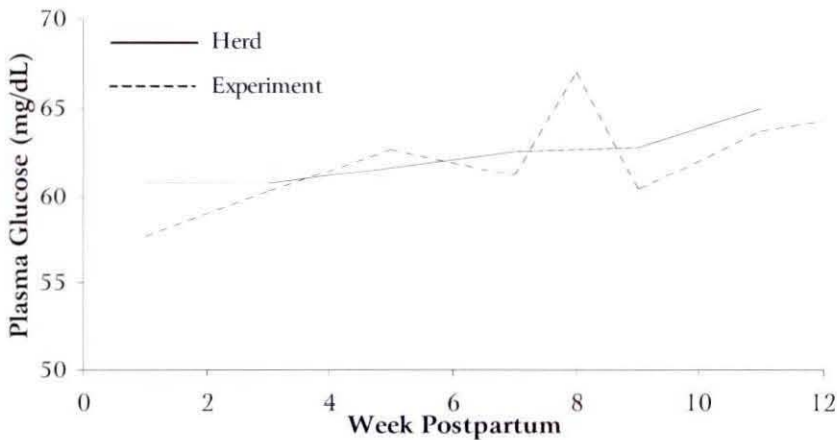

Figure 50. Plasma glucose concentration in the herd

1.3 Metabolic tests

Results of the insulin challenge represent differences in the peripheral uptake of glucose in response to exogenous insulin into insulin-sensitive tissues (Sechen *et al.*, 1989). A rapid decrease in glucose concentration over the first 40 to 45 minutes of the challenge was followed by a steady increase toward baseline concentrations up to 180 minutes after insulin injection. The observed decrease in glucose concentration represented a change of more than 30% and the protocol for the hyperglycaemic clamp was not commenced until the glucose concentration of whole-blood stabilized after the insulin challenge.

Results of the hyperglycaemic clamp represent differences in reaction to endogenous insulin secretion and the steady-state disappearance of glucose in the assumed absence of endogenous glucose production (Weekes *et al.*, 1983, Sano *et al.*, 1991). During the experimental protocols the circulating glucose concentration was rapidly increased over the first 30 minutes of the clamp. Once hyperglycaemia (+ 50 mg/dL whole-blood) was achieved concentrations were maintained within a 10% range in all clamps, at all time points. In one clamp a single observation was 0.4 mg/dL greater than the upper limit, but

this value was equal to the upper limit after rounding and was not considered outside the acceptable range.

The GIR estimates glucose utilization *vs.* endogenous production (i.e. turnover) in the glucose clamp, where hepatic output should be minimal. The GIR during the hyperglycaemic clamp is a measure of the response of tissues to endogenous insulin secretion, as it is proportional to the disappearance of infused glucose from the general circulation (Blum *et al.*, 1999). However the GIR over the entire clamp is confounded by the very similar weight of glucose infused during the attainment of hyperglycaemia and was very similar between all the clamps as expected. The GIR of the last 40 minutes of the clamp, after steady-state conditions are reached (SSGIR) therefore gave a more accurate measure of glucose utilization in response to endogenous insulin secretion.

2. Recombinant bovine somatotropin

2.1 The insulin challenge

Treatment of early lactation dairy cows with recombinant bST decreased the fasting whole-blood glucose concentration by 9.4% of control (-4.6 mg/dL, $P < 0.0001$), which was very similar to the results obtained from the AUC data where the reduction was 9.5% (-139.8 mg × min/dL, $P < 0.0006$). For the fasting plasma glucose concentration a reduction of 10.0% was observed (-6.7 mg/dL, $P < 0.0001$) and plasma baseline AUC decreased by 10.2% (-205.7 mg × min/dL, $P < 0.0006$) in response to recombinant bST treatment. This decrease in glycaemia in response to exogenous somatotropin administration was unexpected, as circulating glucose concentration generally remained unaffected by various bST treatment protocols (Peel *et al.*, 1981, Sechen *et al.*, 1990), with only small (Adriaens *et al.*, 1992) or non-significant (McDowell *et al.*, 1987b, McGuire *et al.*, 1992) increases observed occasionally. It was therefore most likely that the reduction in the rbST period was a remnant of the relative hyperglycaemia observed in the control period, rather than an effect of somatotropin administration.

The differences between the responses during insulin challenge became evident when the data was presented as a percentage of the baseline concentration (Figure 51). The corrected AUC during the first 30 minutes following insulin injection was increased by 26.7% (+62.9 mg × min/dL, P < 0.0107) for whole-blood and by 27.7% (+92.5 mg × min/dL, P < 0.0036) for plasma. These values for the decrease in the glucose response to insulin injection were strikingly similar when one considers that the whole-blood values were calculated from 4 trapeziums, whereas plasma values were calculated from a single data point. The relative unresponsiveness of glucose to exogenous insulin injection was also highlighted by the 21.7% (–4.0 mg/dL, P < 0.0038) reduction in the maximum glucose response, which took 6.5 minutes (+15.5%, P < 0.0037) longer to attain in cows treated with recombinant bST.
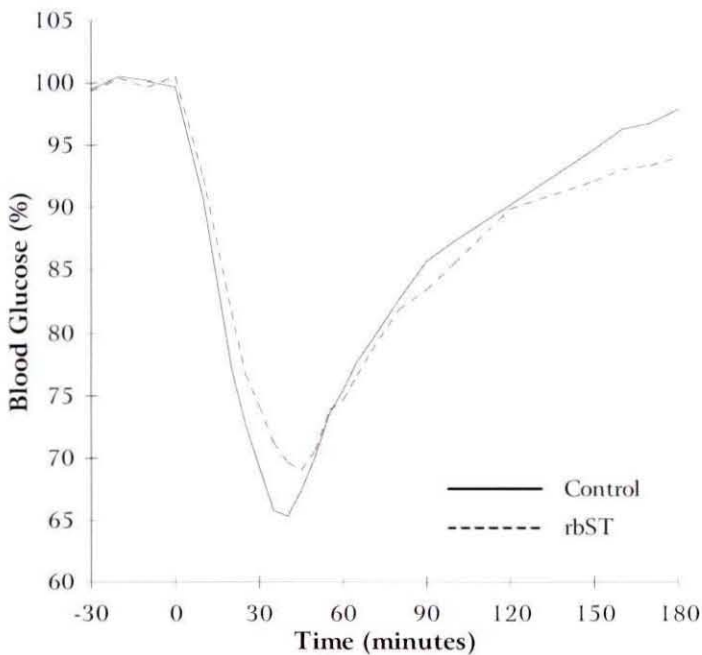

Figure 51. The effect of rbST on insulin challenge results

These results are in agreement with the effects of pituitary-derived bST in early lactation, where a tendency (P < 0.10) toward a 20.5% reduction in total AUC and a 30.8% reduction in the rate of glucose fall was observed during insulin challenge (Sechen et al., 1989). A decrease in the AUC from the time of insulin injection to 30 minutes into the challenge of 32.4%, with a 41.4% decrease in the rate of decline were also observed in experiments using recombinant bST later in lactation (Sechen et al., 1990). It seemed

therefore that the relative hyperglycaemia of the control period did not unduly compromise the results of the insulin challenge in the rbST period in this experiment.

The results of hyperinsulinaemic euglycaemic clamp at peak lactation in Holstein cows were generally unaffected by growth hormone-releasing hormone treatment, whereas decreased GIR, glucose disposal and metabolic clearance rate in response to insulin was evident in late lactation cows (Rose *et al.*, 1996). In wethers there was a 20 to 30% reduction in the GIR during hyperinsulinaemic euglycaemic clamps, decreasing the responsiveness of tissues to insulin resulting in decreased metabolic clearance rate of glucose in response to insulin (Rose & Obara, 1996). In pigs the administration of pituitary-derived (Wray-Cahen *et al.*, 1993) or recombinant pST (Kerber *et al.*, 1998) failed to alter insulin concentration, but increased circulating glucose concentrations and similar to dairy cows the insulin response to challenge remained unaffected while glucose response area and fractional removal rate was lower in somatotropin treated animals. The SSGIR required to maintain euglycaemia during hyperinsulinaemic clamp was also decreased by pituitary-derived pST, where baseline insulin and glucose concentrations were increased by treatment (Wray-Cahen *et al.*, 1993).

2.2 The hyperglycaemic clamp

The effect of recombinant bST treatment on glucose concentration of the second baseline period before the hyperglycaemic clamp was very similar to the fasting baseline preceding insulin challenge, but slightly more accentuated. The average whole-blood glucose concentration was decreased by 12.5% (–6.0 mg/dL, $P < 0.0001$) and the plasma glucose concentration by 14.9% (–9.7 mg/dL, $P < 0.0001$) in cows treated with recombinant bST compared to control. The rate of glucose infusion to maintain hyperglycaemia in the plateau phase (SSGIR) was decreased by 8.1% (–0.2 mg/kg × min, $P < 0.0001$) in the rbST period, which suggests a reduction in the peripheral uptake and/or utilization of glucose from the circulation, in response to increased endogenous insulin secretion (Figure 52). These results are indicative of the ability of exogenous somatotropin to enhance homeorhetic adaptations or nutrient partitioning during early lactation (Bauman & Currie, 1980).

The SSGIR to maintain hyperglycaemia (2.3 mg/kg×min in control) was greater than the rate in dry nonpregnant beef cows (1.8 mg/kg×min), where lactation was characterized by a 44.4% increase in SSGIR to 2.6 mg/kg×min (Sano *et al.*, 1991), or a 37.0% reduction in dairy cows (Sano *et al.*, 1993). The SSGIR was lower than the 3.0 to 3.2 mg/kg×min for cows with a greater milk production at week 9 or 19 of lactation, indicative of greater utilization by the mammary gland (Blum *et al.*, 1999). Administration of exogenous somatotropin to early lactation cows did not significantly affect the glucose, insulin or glucagon responses to glucose challenge (Sechen *et al.*, 1989), with a tendency ($P<0.09$) toward a 34.9% decrease in peak insulin concentration (Adriaens *et al.*, 1992). During glucose challenge the glucose half-life was increased by pituitary-derived pST administration (Wray-Cahen *et al.*, 1993) and the glucose clearance rate was significantly decreased by either pituitary-derived pST or recombinant pST to administration in growing barrows (Gopinath & Etherton, 1989b) coupled to increased insulin responses (Gopinath & Etherton, 1989b, Wray-Cahen *et al.*, 1993).
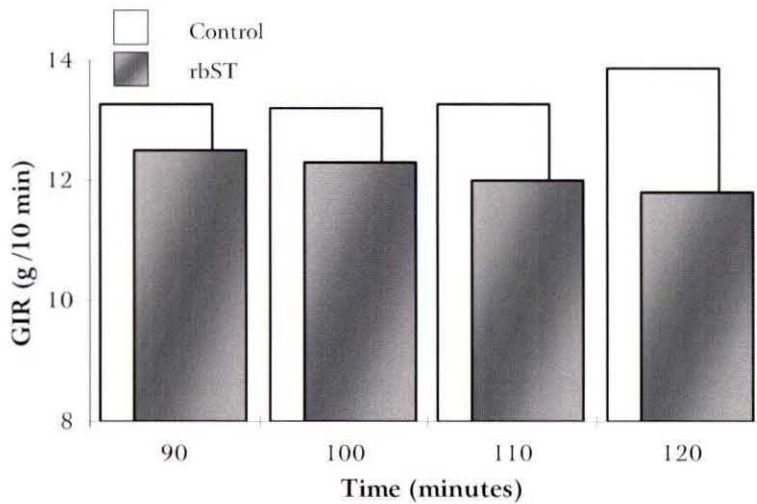

Figure 52. The effect of rbST on GIR in the steady-state period

3. Energy restriction

3.1 The insulin challenge

Restricting the intake of cows from 10 weeks postpartum decreased the availability of glucose compared to control, as illustrated by the 6.2% decrease in the fasting glucose concentration in whole-blood (−3.0 mg/dL, $P < 0.0001$) and 4.9% decrease in plasma (−3.3 mg/dL, $P < 0.0001$). Similarly the baseline AUC was decreased by 6.2% in whole-blood (−91.2 mg × min/dL, $P < 0.0095$) and by 5.0% of control in plasma (−100.5 mg × min/dL, $P < 0.0494$). The effects of nutrient restriction on glycaemia depend on the severity and duration of the restriction and data was consistent with previous reports on restricted intake. Nutrient restriction to 80% of the calculated requirements for net energy and/or crude protein for 8 to 12 days failed to significantly affect the circulating glucose concentration in mid to late lactation cows, where IGF-I was unaffected and insulin decreased by 46.2% (McGuire *et al.*, 1992). Postruminal nutrient infusion of early lactation cows did not affect glycaemia (Peel *et al.*, 1982), while feed deprivation in midlactation decreased circulating glucose (McGuire *et al.*, 1995a). Nutrient restriction in dairy ewes lead to a small decrease (−1.6%) in blood glucose concentration (Metcalf & Weekes, 1990), with a 15.2% reduction in glycaemia in dry and pregnant ewes exposed to more severe nutrient restriction (Petterson *et al.*, 1993). In early lactation Danish Holstein cows intake of <25% lower net energy in early lactation resulted in elevated somatotropin concentration in the face of decreased IGF-I, decreased plasma glucose concentration by 7.6% and insulin by 46.3% (Andersen *et al.*, 2004). The decrease in circulating glucose concentration in response to nutrient restriction of experimental cows could therefore have been a result of the relatively high glucose concentrations recorded during the control period in week 8 postpartum.

None of the glucose responses were significantly affected by restriction of energy intake, as illustrated in Figure 53, where results were corrected for baseline concentrations. Neither whole-blood glucose response to insulin injection ($P < 0.4485$), nor the plasma response ($P < 0.9779$) was affected by energy restriction. This lack of an effect on the glucose uptake into peripheral tissues was confirmed by a lack of effect of treatment on the maximum glucose response ($P < 0.7449$) and the time to reach the minimum whole-blood glucose concentration ($P < 0.7976$).
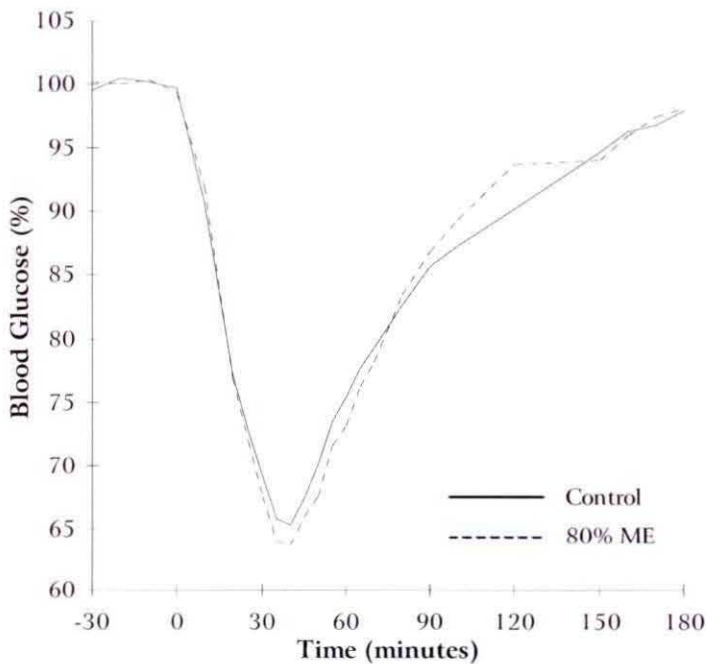
Figure 53. The effect of 80% ME on insulin challenge results

The cows in this experiment were already in the negative energy balance of early lactation and a lack of effect of 20% energy restriction on insulin responses was not surprising. Previous reports of more severe nutrient restriction or more extended periods of treatment have reported significant effects of nutrient supply on the metabolic responses of glucose to insulin, but were generally equivocal. In pregnant ewes significant nutrient restriction (50%) failed to affect whole-body glucose utilization, metabolic clearance rate or insulin-dependent glucose utilization during hyperinsulinaemic euglycaemic clamps, but decreased gluconeogenesis (Petterson *et al.*, 1993). Although basal responses were not affected in dry and lactating ewes, there was a reduction in the sensitivity of glucose metabolic clearance rate, with no change in responsiveness (Metcalf & Weekes, 1990). Because glucose utilization was unaffected by energy restriction, the decline in circulating glucose concentrations can be attributed to the decreased in the alimentary supply of glucose precursors and a subsequent decrease in hepatic glucose output (Petterson *et al.*, 1993). In growing wethers a reduction in nutritional quality (maize-based *vs.* grass-based diet) that failed to affect basal glycaemia or insulinaemia, did not affect the basal clearance of glucose from the circulation or the whole-body glucose metabolism, but decreased glucose clearance in response to euglycaemic hyperinsulinaemia (Janes *et al.*, 1985).

3.2 The hyperglycaemic clamp

Similar to the first baseline period the whole-blood and plasma glucose concentrations were decreased by 4.1% (–2.0 mg/dL) and 4.8% (–3.1 mg/dL) respectively (P < 0.0001). The SSGIR to maintain hyperglycaemia tended to increase (P < 0.0774) by only 0.04 mg/kg × min or 1.7%, an effect that seems of little biological importance (Figure 54). Similarly there was no difference between the GIR at week 9 *vs*. 19 of lactation in high-production dairy cows, where stage of lactation would have a similar nutrient limiting effect due to the difference in milk production (Blum *et al*., 1999). The differences in GIR between the dry period and lactation were equivocal in beef (Sano *et al*., 1991) and dairy cows (Sano *et al*., 1993) and failed to reach statistical significance (P<0.10).
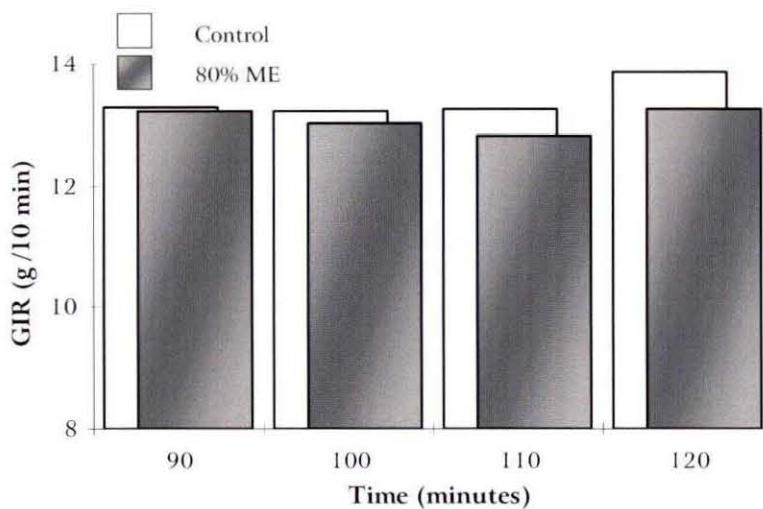

Figure 54. The effect of 80% ME on glucose clamp results

4. Recombinant BST in the face of energy restriction

4.1 The insulin challenge

When energy intake was restricted during treatment with recombinant bST, the whole-blood glucose concentration was 2.4 mg/dl greater (+5.5%, P < 0.0001) and the plasma glucose concentration 3.9 mg/dL greater (+6.5%, P < 0.0001), but not increased to the level of the control period. The fasting baseline AUC for whole-blood tended to increase by 5.5% (+73.1 mg × min/dL, P < 0.0571) compared to the rbST period and reached

statistical significance when plasma AUC were compared, which increased by 6.6% (+119.4 mg × min/dL, $P < 0.0313$). Whereas nutrient restriction was characterized by decreased availability of glucose due to a reduction in alimentary substrates (Petterson *et al.*, 1993), exogenous bST administration resulted in a greater availability of glucose due to nutrient partitioning effects (Bauman *et al.*, 1988). These contradictory effects on glucose metabolism could have contributed to glucose concentrations that were intermediate between the control and rbST periods when treatments were combined.

Combination with energy restriction did not affect the corrected response AUC of either whole-blood or plasma compared to recombinant bST treatment alone (Figure 55). The maximum glucose response of the 80% ME + rbST period tended to be 17.1% greater than the rbST period ($P < 0.0646$), but was also not different from control. There was no effect of energy restriction on the recombinant bST effect on the timing of the maximum glucose response ($P < 0.2126$). The somatotropin resistance induced by energy restriction (Andersen *et al.*, 2004) and decrease in the number of somatotropin receptors (Newbold *et al.*, 1997) can result in a reduction in the biological efficacy of recombinant bST treatment. From these data it is clear that combination with nutrient restriction blunted some, but not all of the glucose responses to recombinant bST administration in early lactation dairy cows, where many of the responses to exogenous and endogenous insulin were intermediate between the two treatments. This could be ascribed to the decrease in alimentary substrates and a reduction in the insulin-independent utilization of glucose (Petterson *et al.*, 1993), which would include utilization by the mammary gland when milk production decreased.
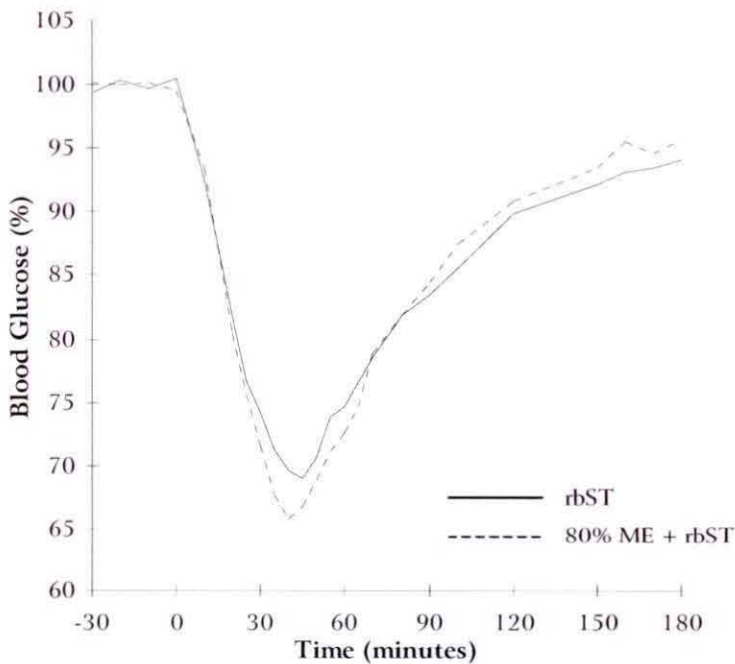
Figure 55. The effect of 80% ME on rbST insulin challenge results

4.2 The hyperglycaemic clamp

Compared to recombinant bST treatment on an *ad libitum* intake regime, administration during nutrient restriction yielded very similar results to the first baseline period with an increase in concentration, but not to the level of controls. The whole-blood glucose concentration was increased by 7.2% (+3.0 mg/dL) and the plasma glucose concentration by 10.8% (+6.0 mg/dL) of control (P < 0.0001). The SSGIR of the 80% ME + rbST period (Figure 56) was 0.1 mg/kg × min, or 5.1% greater than the rbST period (P < 0.0005), but still decreased compared to the control period (P < 0.0133). The greater need for glucose infusion is consistent with the tendency toward a greater glucose maximum response and is indicative of a greater use of glucose by tissues. An increase in glucose oxidation in peripheral tissues (Rhoades *et al.*, 2007) and reduction in gluconeogenesis (Petterson *et al.*, 1993) are in direct opposition to the effects of somatotropin treatment (Bauman *et al.*, 1988). Full development of the galactopoietic response relies on high quality nutrient supply to increase the amount of hepatic binding sites for somatotropin, the normal responses of the liver and IGF-I concentration (Newbold *et al.*, 1997). Nutrient deprivation and/or nutrient restriction modulated the normal responses within the somatotropic axis, decreasing the trophic effects of exogenous somatotropin and effects on binding proteins (McGuire *et al.*, 1995a). Combination of

glucose and casein supplementation with pituitary-derived bST treatment failed to elicit responses beyond that of somatotropin alone (Peel *et al.*, 1982). In cows in later lactation where excess nutrient supply was compared to restriction of net energy and/or crude protein to 80% of calculated requirements, no interaction between dietary treatments and recombinant bST administration was observed (McGuire *et al.*, 1992).
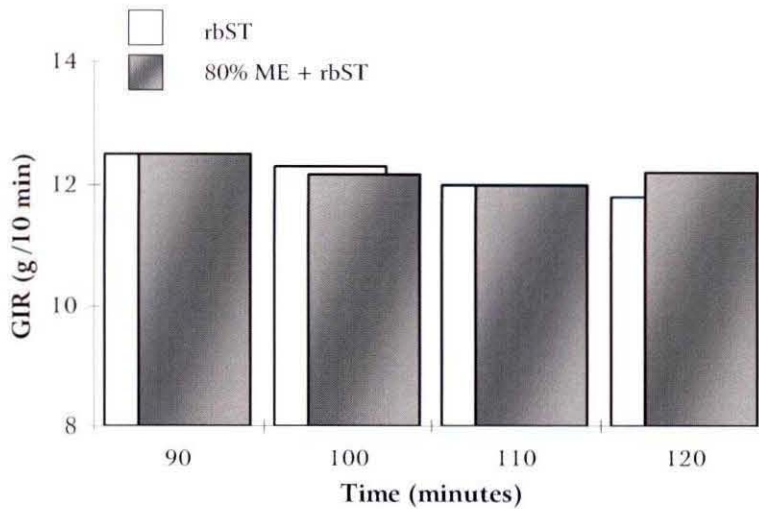

Figure 56. The effect of 80% ME on rbST glucose clamp results

The administration of recombinant bST in the face of inadequate nutrition is coupled with an absence of an IGF-I response due to the nutritional effect on hepatic somatotropin receptors, which decrease in response to a negative energy balance (reviewed by Breier, 1999). While recombinant bST alone decreased glucose utilization under *ad libitum* conditions, the nutrient partitioning effects of exogenous somatotropin were decreased by nutrient restriction. The increase in glucose concentration cannot be explained by a change in the alimentary supply or peripheral utilization of glucose, both of which changed in the opposite direction. The most likely source of the "excess" circulating glucose is reduced utilization for lactose synthesis by the mammary gland and might be explained by the 1.7 kg/d (−6.1%) decrease in milk production that seemed to occur between week 10 and 12.

5. Energy restriction coupled to rbST administration

5.1 The insulin challenge

Compared to energy restriction alone (80% ME), the addition of recombinant bST treatment (80% ME + rbST) did not affect the parameters of fasting glucose concentration to any great extent. There was a small (+1.8%) but significant increase in the blood glucose concentration (P < 0.0001). The whole-blood response AUC, the maximum glucose response and time to reach this response were unaffected by the administration of recombinant bST in the face of nutrient restriction. The glucose response AUC in plasma only tended to reach statistical significance (P < 0.0734) and constituted a 17.4% (+57.9 mg × min/dL) reduction in the reaction to insulin injection (Figure 57), suggesting that recombinant bST treatment was still able to induce mild alterations in partitioning within the intake restricted cows.
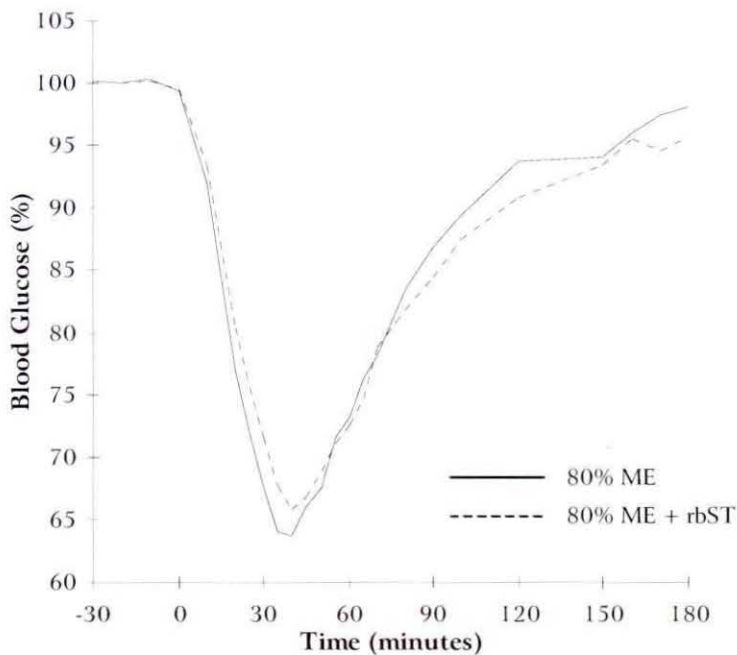

Figure 57. The effect of rbST on 80% ME insulin challenge results

5.2 The hyperglycaemic clamp

Recombinant bST administration lead to a 1.0 mg/dL decrease in the whole-blood glucose concentration preceding hyperglycaemic clamp, but like the fasting concentration difference this effect was extremely small (-2.2%, $P < 0.0001$). The baseline plasma concentration remained unaffected by treatment ($P < 0.2356$). However, the GIR to maintain hyperglycaemia in the steady-state phase of the clamp was decreased by 5.0% (-0.1 mg/kg $\times$ min, $P < 0.0004$) in the 80% ME + rbST period compared to the 80% ME period (Figure 58), resulting in a response intermediate between the control and rbST periods. Combining recombinant bST administration did not alter the effects of nutrient restriction on responses to exogenous insulin challenge and although a slight improvement of the hypoglycaemia of energy restriction can be suggested, these effects were too small and inconsistent to be of importance. The decrease in SSGIR is consistent with a sparing effect of recombinant bST treatment on glucose utilization, even in the face of an already-negative energy balance. This "extra" glucose could not arise from alimentary sources and there must have been a reduction in peripheral utilization or the synthesis of lactose, as there seemed to be a pronounced decrease in milk production between week 11 and 12 of 2.1 kg/d or 7.4%. The effect observed in the hyperglycaemic clamp in the absence of an effect in the insulin challenge can be explained by differences in the endogenous secretion of insulin.
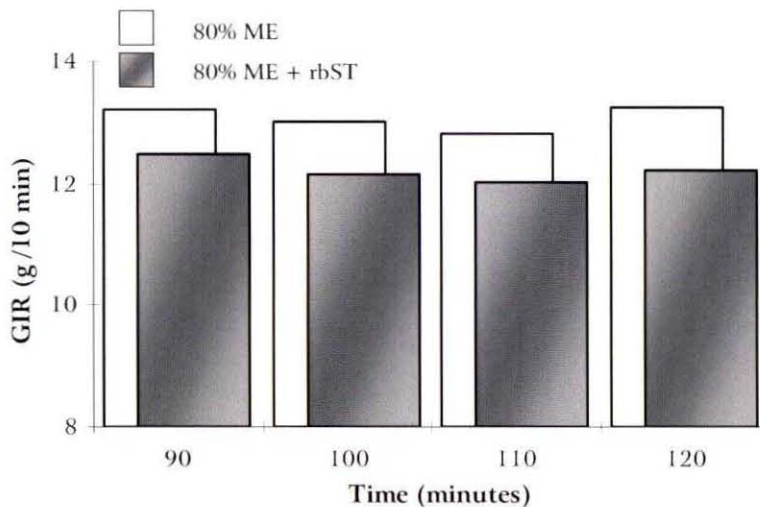

Figure 58. The effect of rbST on 80% ME glucose clamp results

CHAPTER 6. CONCLUSION AND CRITICAL EVALUATION

The most notable effects on glycaemia and glucose responses to exogenous or endogenous insulin were observed for the recombinant bST treatment period, while glycaemic and not metabolic responses were evident in the intake restricted treatment period. Responses to insulin challenge were generally attenuated when treatments were combined and intermediate between treatment and control. Whereas some of the responses to recombinant bST were completely reversed by nutrient restriction and some like glucose utilization in response to endogenous hyperinsulinaemic were not increased to the level of control, others like glucose response area and the timing of the maximum glucose response remained unchanged. Therefore, even in the early lactation dairy cows where extensive homeorhetic adaptations are already at work, recombinant bST administration modulated glucose metabolism to spare more glucose, even in the face of nutrient restriction.

A negative energy balance in the early lactation, high-production cow is inevitable, while energy supplementation has the ability to negatively affect the protein supply to metabolic processes, especially through altered conditions in the rumen. Optimizing the state of energy and protein metabolism in the face of this negative energy balance is imperative and requires a detailed understanding of the metabolic status of early lactation dairy cows. Administration of recombinant bST has the ability to enhance the synthesis of milk components, where the lipid content of milk can be enhanced in this early lactation period, but care should be taken in the protein status of animals to prevent a reduction in milk protein content. These effects occur through enhancement of the homeorhetic adaptive responses of nutrient partitioning and body reserve mobilization that are also the principal means by which productive efficiency of cows can be enhanced (Bauman *et al.*, 1985b).

Originally the experiment was envisaged as a completely randomized design using 40 cows that would ideally be subjected to hyperinsulinaemic euglycaemic clamps and hyperglycaemic clamps involving markers during control and treatment periods. Due to constraints in herd size, funding, time and equipment (resources) it was decided that insulin challenges and hyperglycaemic clamps without the use of markers could still yield

useful results from a smaller number of animals where treatments could be combined. Although complete randomization of treatments would then not be possible, a randomized crossover design was pictured, where the effects of treatments on the metabolic tests, homeorhetic hormones and their interactions would be analyzed. We were advised to apply all treatments to all cows to accommodate the limits within which the research was conducted and that a crossover Latin square would allow too great a variation in the physiological status of cows within the very dynamic early lactation period. Subsequently each cow received each treatment in the same sequence at approximately the same stage of lactation. In hindsight this decision could have been responsible for the apparent confounding effects of the control period and the altered physiology between week 8 to 12 of lactation could have been acceptable within the scope of the research. Randomized crossover would accommodate this variation in physiological state where application of two instead of all four treatment periods could have been a viable alternative to decrease the length of the experimental period and the number of cows used.

Ideally the sample analyses of plasma would have included assays of IGF-I, insulin and leptin. We were assured that peptide integrity would be maintained for the duration of the experiment as long as the samples did not undergo freeze-thaw cycles, even though assay manuals clearly stated the need to analyze samples shortly after collection. The convention of the laboratories was to complete the experiments and subsequently determine which samples to analyze following the practical aspects of the research. This period proved too long and several months elapsed between sample collection and assay, resulting in peptide loss (Chapter 3, section 6). It is recommended that occasional sample analyses should be conducted throughout the application of metabolic tests when experimental periods are extensive, especially for samples intended for assay of insulin-like growth factors. Although leptin was confirmed to be stable even when subject to repeated freeze-thaw cycles (Flower *et al.*, 2000), a commercial kit was advertized but not yet available at the time of sample analyses. During the planning phase of the research the role of leptin in ruminant animals and homeorhesis was only emerging (Zang *et al.*, 1994) and it was assumed that the assay techniques applied to human samples could accurately estimate bovine leptin due to peptide homology (Zang *et al.*, 1997). It later became clear

that, although bovine leptin was able to bind antibodies of a mutispecies leptin kit (Minton *et al.*, 1998), the assay seemed inadequate for application in domestic ruminants (Ehrhardt *et al.*, 2000) as there seemed to be interference of plasma components with the assay with inconsistent variation in results, probably due to differences in peptide folding (Devalaud *et al.*, 2000).

Although the data available from the metabolic tests were limited by the failure to determine some of the intended hormones, valuable data was still collected from the metabolic tests. Protocols were generally applied successfully and the data on glycaemic responses yielded useful results. In addition to glycaemic responses to insulin challenge and endogenous insulin secretion, the NEFA response to epinephrine challenge as the other major response to recombinant bST treatment could be included to attain a more complete picture of metabolic adaptations. The data could also be improved by the combination of assay for other homeorhetic responses during lactation, for example leptin and somatotropin concentration responses to treatments, where the correlation and/or interaction of glycaemic responses could be determined.

Although somatotropin is the most important homeorhetic hormone of lactation, future research can explore the importance of other hormonal responses during lactation that determine metabolic adaptations. The aim would be to establish enhanced mobilization responses within the limits of adaptation to ensure that metabolic imbalances or metabolic diseases do not increase significantly. The metabolic adaptations of lactation for glucose and lipids have been explored at a general and molecular level in some detail and potential factors for enhancement of protein and amino acid responses to express milk production fully (especially milk protein production) are possibilities for future research.