Circulating glucose responses in early lactation dairy cows to dietary restriction and rbST treatment

by

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BSc (Agric) Animal Science

Submitted in fulfilment of the requirements of the degree
MSc (Agric) Animal Science: Production Physiology in the
Faculty of Natural and Agricultural Sciences
University of Pretoria

June 2008

I declare that the thesis, which I hereby submit for the degree MSc (Agric) Production Physiology at the University of Pretoria, is my own work and has not previously submitted by me for a degree at this or any other tertiary institution.

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ABSTRACT

Galactopoietic effects of somatotropin are the result of IGF-I and require high-quality nutrient intake. This study investigated short-term partitioning effects during recombinant bovine somatotropin (bST) administration in high yielding early lactation dairy cows. Administration of recombinant bST has been shown generally to alter results of metabolic tests in the face of unchanged basal glucose and insulin concentrations. Ten multiparous Holstein cows were subjected to rbST (Lactotropin®) and/or feed intake restriction to 80% of predicted ME requirement (80% ME). Responses to insulin challenge (0.1 IU porcine insulin/kg BW, 210 min) and hyperglycaemic clamp (+50 mg/dL whole blood, 120 min) were tested during weeks 8 (control), 9 (rbST), 11 (80% ME) and 12 (rbST + 80% ME) postpartum. Plasma and whole blood samples were assayed for glucose concentrations. The rbST treatment decreased fasting whole-blood glucose concentration by 9.4% ($P < 0.0001$), which was likely a remnant of control hyperglycaemia. Maximum glucose response was 4.0 mg/dL (21.7%) lower ($P<0.0038$) and took 6.5 minutes longer to attain ($P<0.0037$). Steady-state glucose infusion rate (SSGIR) decreased by 8.1% ($P<0.0001$). The 80% ME treatment decreased glucose availability by 5 to 6% ($P<0.0100$), while no glucose responses were affected. Restricted energy intake during treatment with rbST resulted in plasma glucose increase by 5.5% ($P<0.0001$). Peripheral uptake and utilization of glucose increased by 5.1% ($P<0.0005$). Compared to energy restriction, 80%ME + rbST did not alter effects of nutrient restriction on responses to exogenous insulin challenge. Effects were small and inconsistent. SSGIR decreased by 5.0% in the 80% ME + rbST compared to the 80% ME period ($P<0.0004$) and the change in the hyperglycaemic clamp in the absence of an effect in the insulin challenge may be due to differences in endogenous insulin secretion. The conclusion was that rbST treatment resulted in altered glucose metabolic responses, even with restricted energy intake.

Keywords

insulin resistance	glucose clamp	recombinant bST
early lactation	Holstein cows	nutrient restriction

Acknowledgements

I want to thank my supervisor Prof. N. H. Casey for his valued contribution and patience during the completion of this project. Many thanks to all the University of Pretoria Hatfield experimental farm Dairy Unit personnel who were always ready with a helping hand and advice. Statistical and data processing assistance provided by Prof. H. Groeneveld and R. Coertze is gratefully acknowledged. This research was supported by funding from the Department of Animal and Wildlife Sciences, University of Pretoria (Professor N. H. Casey).

SUMMARY

The 9.4% reduction in glycaemia induced by recombinant bST in this experiment was uncharacteristic and a residual effect of a relative hyperglycaemia in the control period. Administration of slow-release recombinant bST in week 8 of lactation resulted in a reduction of the insulin-induced glucose responses at 7 days of treatment similar to previous experiments in dairy cows at 9 weeks (Sechen *et al.*, 1989) and 27 weeks postpartum (Sechen *et al.*, 1990), although the reduction in insulin biological effect was less pronounced early in lactation, probably because milder homeorhetic adaptations were required later in lactation. The responses to recombinant bST included a reduction in response area and the size of the maximal response, while delaying the attainment of maximal reduction in glucose in response to insulin challenge. There was a reduction in glucose disposal in tissues in response to hyperinsulinaemia, where decreased utilization of glucose in response to insulin would make more glucose available to tissues that do not depend on insulin for glucose uptake, like the lactating mammary gland. Similarly the GIR of exogenous hyperinsulinaemic euglycaemia was decreased in lactating cows at 28 weeks postpartum (Rose *et al.*, 1996) and in growing wethers (Rose & Obara, 1996), which was indicative of a decreased utilization of glucose in response to insulin in peripheral tissues. An increase in glucose availability induced by decreased oxidation (Bauman *et al.*, 1988) and increased gluconeogenesis (Pocius & Herbein, 1986, Knapp *et al.*, 1992) could at least in part explain the reduction in SSGIR in response to endogenous insulin release and data confirm the role of exogenous somatotropin in enhancing the nutrient partitioning effects of homeorhetic adaptations that were already at work in these early lactation dairy cows.

The mild nutrient restriction resulted in reduced circulating glucose concentration that could have been the result of the relative hyperglycaemia of the control period, but could also be the result of sustained milk production under conditions where gluconeogenesis was decreased in the face of a reduction in alimentary substrates (Petterson *et al.*, 1993). Whereas similar reductions in ME and/or crude protein intake in dairy cows failed to affect glycaemia (McGuire *et al.*, 1992), feed deprivation (Peel *et al.*, 1982) or net energy restriction of more than 25% (Andersen *et al.*, 2004) was required to decrease circulating glucose concentration in cows. There was no effect of restriction on the glucose responses to exogenous or endogenous insulin during the insulin challenge or hyperglycaemic clamp.

The small tendency toward increased glucose utilization in response to insulin ($P<0.0774$) seemed of little biological importance. Many of the glucose metabolic responses to insulin failed to respond to even greater nutrient restriction in ewes (Metcalf & Weekes, 1990, Petterson *et al.*, 1993) and wethers (Janes *et al.*, 1985), or to variation in nutrient supply or physiological status in beef (Sano *et al.*, 1991) or dairy (Sano *et al.*, 1993, Blum *et al.*, 1999) cattle.

The ability of recombinant bST to modulate the glucose responses to insulin was not significantly affected by combination with nutrient restriction. The slightly higher glucose concentration could be the result of altered nutrient partitioning and a decrease in utilization of glucose by the mammary gland (Petterson *et al.*, 1993). However the amount of glucose disposal in response to elevated endogenous insulin was higher in the combined treatment period than recombinant bST alone, indicative of failure of the full development of the recombinant bST response on the restricted intake regimen. Similarly there was a tendency for the maximum response of glucose to insulin challenge to be increased ($P<0.0646$) toward levels that were not different from control ($P<0.4071$), i.e. nutrient restriction tended to completely prevent the response to recombinant bST. The continued response to recombinant bST in the face of nutrient restriction could also be related to the fact that the uncoupling of the somatotropic axis during undernutrition (Newbold *et al.*, 1997) reduced the indirect effects of somatotropin through the IGF system (McGuire *et al.*, 1992, McGuire *et al.*, 1995a) and could also involve the direct metabolic effects of somatotropin through tissue somatotropin resistance (Breier, 1999). Generally recombinant bST failed to significantly affect the glucose responses to insulin under a restricted intake regimen. The application of recombinant bST had inconsistent, but very small effects on the hypoglycaemia of nutrient restriction. Although nutrient restriction alone failed to affect the disposal of glucose in response to endogenous hyperinsulinaemic euglycaemia, combination with recombinant bST decreased glucose utilization, but not to the same extent as recombinant bST alone. Even apparently small or non-significant effects of nutrient intake on the ability of recombinant bST to induce altered nutrient partitioning (Peel *et al.*, 1982, McGuire *et al.*, 1992) could still have far-reaching effects on the supply of nutrients to the lactating mammary gland, leading to large reductions in the ability to modulate production responses (McGuire *et al.*, 1992, Newbold *et al.*, 1997).

Summary of data collected during the experimental period

Whole-blood	Control	rbST	80% ME	80% ME+ rbST
Insulin challenge:				
Baseline glucose concentration (mg/dL)	[a] 48.7	[b] 44.1	[c] 45.7	[d] 46.6
Baseline AUC (mg×min/dL)	[a] 1464.2	[b] 1324.4	[b] 1373.0	[ab] 1397.6
Response AUC (mg×min/dL)	[a] –235.7	[b] –172.8	[ab] –218.9	[ab] –190.3
Maximum response (mg/dL)	[a] 18.3	[b] 14.3	[a] 17.9	[ab] 16.8
Time to maximum (min)	[a] 42.0	[b] 48.5	[ab] 42.5	[b] 46.0
Glucose clamp:				
Baseline glucose concentration (mg/dL)	[a] 47.5	[b] 41.5	[c] 45.5	[d] 44.5
Total GIR (mg/kg×min)	[a] 2.8	[a] 2.8	[a] 2.9	[a] 2.9
SSGIR (mg/kg×min)	[a] 2.3	[b] 2.1	[a] 2.3	[c] 2.2

Plasma	Control	rbST	80% ME	80% ME+ rbST
Insulin challenge:				
Baseline glucose concentration (mg/dL)	[a] 67.1	[b] 60.4	[c] 63.8	[c] 64.3
Baseline AUC (mg×min/dL)	[a] 2016.2	[b] 1810.5	[c] 1915.7	[ac] 1929.9
Response AUC (mg×min/dL)	[a] –333.8	[b] –241.3	[ac] –333.1	[bc] –275.2
Glucose clamp:				
Baseline glucose concentration (mg/dL)	[a] 65.0	[b] 55.4	[c] 61.9	[c] 61.4

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods

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SYMBOLS AND ABBREVIATIONS

% $^{w}/_{v}$	-	weight per volume percentage
80% ME	-	the 80% of estimated metabolizable energy requirement period
80% ME + rbST	-	the combined 80% ME and rbST treatment period
°C	-	degrees Celsius (centigrade)
A_{450}	-	absorbance at 450 nm
AUC	-	area under the (response) curve
BCS	-	body condition score
BP	-	binding proteins
bST	-	bovine somatotropin
C	-	carbon
CoA	-	coenzyme A
CO_2	-	carbon dioxide
CV	-	coefficient of variation
d	-	day
e–	-	electrons
EDTA	-	ethylene diamine tetra-acetic acid
ELISA	-	enzyme-linked immunosorbent assay
g	-	gravitational force (m/s^2)
g	-	gram
$G{\cdot}H_2O$	-	glucose monohydrate
GIR	-	glucose infusion rate
GLUT	-	facilitated diffusion hexose transporter
G protein	-	guanosine triphosphate binding protein, inhibits (Gi) or stimulates (Gs) *adenylyl cyclase*
h	-	hour
H^+	-	hydrogen ions (protons)
H_2O	-	distilled water
H_2O_2	-	hydrogen peroxide
IGF	-	insulin-like growth factor
IGFBP	-	insulin-like growth factor binding protein
IU (U)	-	international units
$IU/kg^{0.75}$	-	international units per kilograms metabolic weight
kg	-	kilogram
L	-	liter
Mcal	-	megacalorie (where 1 calorie is 4.1855 joules)
ME	-	metabolizable energy
mg/dL	-	milligrams per deciliter (100 mL)
mg/kg×min	-	milligrams per kg body weight, per minute
min	-	minute
MJ	-	megajoule (where 1 joule is 0.2389 calories)
mol	-	moles (6.02×10^{23} per mol)
MPII	-	mean plasma insulin increment (of the hyperglycaemic clamp)
nA	-	nanoampere (nanoamps)
NADPH	-	reduced coenzyme (nicotinamide adenine dinucleotide phosphate)
NEFA	-	non-esterified fatty acids
O_2	-	oxygen
P	-	probability value ($P < 0.05$ considered statistically significant)
pST	-	porcine somatotropin
r	-	correlation coefficient
rbST	-	the recombinant bovine somatotropin treatment period
rpm	-	revolutions per minute
SD	-	standard deviation
SSGIR	-	steady-state glucose infusion rate
t_ (t30)	-	time in minutes relative to challenge (e.g. 30 minutes after insulin injection)
TMB	-	tetramethylbenzidine
Zn	-	zinc

CHAPTER 1. INTRODUCTION

Title: Circulating glucose responses in early lactation dairy cows to dietary restriction and rbST treatment

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Very early lactation is the most crucial period in the cow's production cycle, when the already-altered metabolism of pregnancy must supply in an even greater demand for nutrients (Bauman & Currie, 1980), where lactation in the high-producing dairy cow is characterized by further physical, digestive and metabolic adaptations to accommodate the physiological state of the animal (Bell & Bauman, 1997). Homeorhetic adaptations maintain circulating nutrient concentrations to sustain a particular physiological state (like lactation) and supply peripheral tissues with alternative fuels, by altering the physiological responses to homeostatic mechanisms (Bauman & Currie, 1980). Being a large foregut fermenter, altered nutrient metabolism is the most important adaptation to sustain the output of a large volume of high quality product and somatotropin is the most important homeorhetic hormone that repartitions nutrients toward the lactating mammary gland (Bell & Bauman, 1997).

Somatotropin is the principle hormone that coordinates the metabolic responses during the transition from pregnancy into lactation, through major adjustments in nutrient utilization in most body tissues (Bell, 1995). The principal effects of somatotropin are on

adipose tissue lipid metabolism (Bauman & Currie, 1980), through increased responses to lipolytic stimulators and decreased responses to antilipolytic effectors (Doris *et al.*, 1996). Exogenous somatotropin administration altered glucose homeostatic responses to insulin in ruminant animals, affecting the dose-response characteristics with decreased whole-body metabolism and clearance of glucose from the circulation in response to insulin (Rose & Obara, 1996). These changes make more gluconeogenic substrate, glucose and preformed fatty acids available to the mammary gland from body reserves, while the eventual increase in intake of chronic treatment ensure maintenance of the response. Recombinant bST is currently being widely used in the industry as a management tool to increase milk production, beyond the basal genetic capabilities of dairy cows. Although increases of between 10 to 15% were commonly attained, there was no advantage to administering recombinant bST when management, more specifically nutritional management, of the herd was poor (McGuire *et al.*, 1995a). In the latter case, reproductive performance may consequently be depressed leading to overall poor herd performance and profitability (see review by Etherton & Bauman, 1998). The nutritional status of early lactation dairy cows is a crucial factor that determined the nutrient partitioning and milk production responses (Vicini *et al.*, 1991), while nutrient restriction modulated the efficacy of somatotropin within the somatotropic axis (McGuire *et al.*, 1995a).

We therefore hypothesize that somatotropin will enhance homeorhetic responses through altered responses to exogenous and endogenous insulin, even in the early lactation dairy cow at 9 weeks postpartum where metabolism is already altered to accommodate the physiological state. We further suggest that the responses will be modulated by nutrient restriction, where responses may fail to fully express in the face of decreased alimentary nutrient supply.

CHAPTER 2. LITERATURE REVIEW

1. Metabolic tests

1.1 Domestic ruminant *vs.* monogastric animals

Insulin plays a central role in the control of energy metabolism in the body, including substrate distribution to body tissues from the liver (Zammit, 1996), which in turn plays a central role in metabolism through nutrient distribution and modification (Danfær, 1994). The biological effects of insulin, especially in relation to glucose metabolism are indicative of the metabolic state of the animal, with distinctive direct (hepatic) *vs.* indirect (extrahepatic) effects on the utilization and distribution of glucose by the liver (Satake *et al.*, 2002). In addition to differences in circulating concentrations of insulin and glucose in the monogastric animal and the domestic ruminant animal, the control mechanisms that determine glucose homeostasis are very different between species. Sheep are much more resistant to insulin than either humans or pigs and this decrease in homeostatic response to insulin is an important adaptation to ruminant digestion, although insulin still plays a very important role in ruminant glucose homeostasis (Bell & Bauman, 1997). The reduction in insulin action in polygastric compared to monogastric animals has features in common with the changes in physiology during pregnancy and lactation (Bell, 1995), as well as the responses to exogenous somatotropin treatment during growth and lactation. Therefore the relative insulin resistance of the altered physiological state (Petterson *et al.*, 1993) is further augmented by recombinant bST administration (Sechen *et al.*, 1990). Of the homeorhetic hormones, only somatotropin seems to maintain a uniform role in coordinating glucose metabolism to enhance glucose precursor supply and hepatic gluconeogenesis, while decreasing its peripheral utilization (Bell & Bauman, 1997). Hormonal and/or nutrient profiles alone are inadequate to estimate homeostatic or homeorhetic control mechanisms and some form of metabolic test is required to establish the size of tissue responses to hormonal signals (Metcalf & Weekes, 1990).

A whole range of metabolic tests is available to assess the state of the glucose homeostatic mechanisms of the animal, which include exogenous application of glucose, insulin, glucagon and/or epinephrine. Although the application of a single bolus (i.e. a metabolic challenge) is limited in the scope of the conclusions that can be made from the data

collected compared to sequential clamps (Lemosquet & Faverdin, 2001), they are most commonly used in experimental research due to lower costs and ease of application. These tests are usually performed after an overnight fast, because varying period of the feeding cycle can alter glucose metabolism and also the results of metabolic tests, even in the ruminant animal (Sano *et al.*, 1990).

The most complete picture of whole-body insulin-induced glucose metabolism is derived from sequential infusion of varying doses of insulin in the hyperinsulinaemic euglycaemic clamp (Bergman *et al.*, 1985), which often presents the researcher with challenges in the form of high cost and expertise required to perform clamps (Lemosquet & Faverdin, 2001). This technique very accurately distinguishes between the maximum responsiveness *vs.* the half-maximal sensitivity of glucose responses to insulin, as well as glucose appearance *vs.* glucose disposal when coupled to isotope infusion. This information on insulin biological action is improved by combination with hyperglycaemic clamps to assess the pancreatic insulin response to increased circulating glucose concentrations. It is very important to distinguish between the different components of hormone resistance (see Figure 1) and care should be taken in the use of terms like responsiveness (meaning maximum response or R_{max}) and sensitivity (meaning half-maximum response or ED_{50}) in reference to biological actions of hormones (Kahn, 1978).

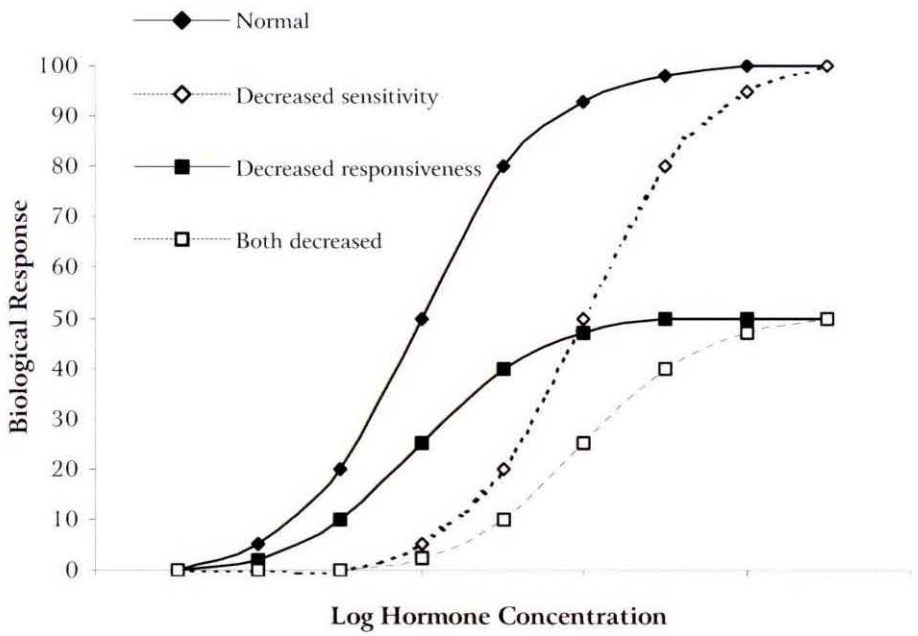

Figure 1. The components of hormone resistance (Kahn, 1978)

Some of the most notable differences between glucose homeostasis in humans *vs.* sheep can be illustrated from results of the first sequential hyperinsulinaemic euglycaemic clamps in humans (Rizza *et al.*, 1981) and the first clamps performed in ruminant animals (Weekes *et al.*, 1983). The basal plasma glucose and insulin concentrations were 96.9 mg/dL glucose and 12 μU/mL insulin in man (Rizza *et al.*, 1981), compared to only 69.6 mg/dL glucose and 5.7 μU/mL insulin in sheep (Weekes *et al.*, 1983). Although generally accepted to reflect differences between polygastric *vs.* ruminant animals, care should be taken as some forestomach fermenters like camels (*Tylopoda*) exhibited a relative hyperglycaemia (128 mg/dL) coupled to reduced insulin biological action compared to sheep (Elmahdi *et al.*, 1997). Although differences in methodology and calculation of results makes comparison between experiments difficult (Bergman *et al.*, 1985), some general features of the differences in insulin action between monogastric *vs.* domestic ruminant animals were apparent.

The steady-state glucose infusion rate (SSGIR) or amount of glucose infusion required to maintain euglycaemia during the final 40 minutes of hyperinsulinaemic euglycaemic insulin infusion is a measure of the whole-body effect of insulin on glucose metabolism. That is the sum of the insulin-induced suppression of glucose output by the liver and stimulation of glucose uptake and utilization by peripheral tissues (Rizza *et al.*, 1981). The SSGIR was consistently greater in man than the infusion rate in sheep, as illustrated in Figure 2. It is clear from the figure that the maximum responsiveness of glucose metabolism to insulin in sheep was greatly decreased compared to humans, with a maximum response to insulin in humans of 10 to 11 mg/kg×min (Rizza *et al.*, 1981), while SSGIR remained below 4 mg/kg×min in sheep (Weekes *et al.*, 1983). However, the insulin concentration for half-maximal effect or sensitivity of whole-body glucose metabolism was similar between species, at an insulin concentration of 58 μU/mL in man (Rizza *et al.*, 1981) and 52 μU/mL in sheep. The maximal responsiveness remained unaffected by undernutrition or altered physiological state (pregnancy) in fed ewes, while the sensitivity of glucose metabolism to insulin was greatly decreased during pregnancy, which was characterized by a reduction in the insulin-dependent glucose utilization (Petterson *et al.*, 1993). Therefore the effect on overall glucose homeostasis is a decrease in responsiveness and not sensitivity of whole-body glucose metabolism in response to insulin in ruminant

vs. monogastric animals. Although a true fasting level of glucose metabolism cannot be attained in the ruminant animal where some digestive products will remain in the gastrointestinal tract, it is important not to compromise data by differing periods of the feeding cycle (Sano *et al.*, 1990).

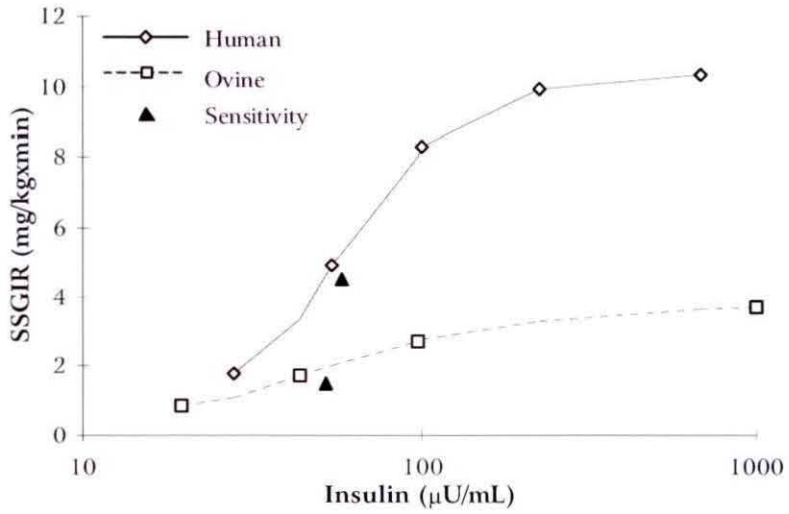

Figure 2. Dose-response curve for SSGIR in humans *vs.* sheep (adapted from Rizza *et al.*, 1981 and Weekes *et al.*, 1983)

From data collected while attempting to eliminate the confounding effects of digestive function in ruminant animals have also noted an additional reduction in the sensitivity of glucose metabolism to insulin. These data were however also often associated with compromising effects associated with lenient application of the protocol of the hyperinsulinaemic euglycaemic clamp. A maximum SSGIR in sheep of 4.9 mg/kg×min has been reported, but euglycaemia was not effectively maintained and SSGIR was increasing between t80 and t120 (Janes *et al.*, 1985). Similar greater glucose infusion rate (GIR) of 4.2 to 4.6 mg/kg×min was quoted in fed sheep, but insulin administration was not preceded by a priming dose and the clamped concentrations lasted only 60 minutes (Sano *et al.*, 1996). The entire period was used in generating curves and not the more correct SSGIR, while carryover effects of a hyperglycaemic clamp just before the clamp was clearly evident in one group (Sano *et al.*, 1996). The results of responsiveness of fasting whole-body glucose metabolism in cattle was calculated as the total glucose entry, including the total GIR of the entire clamp plus endogenous synthesis (Eisemann *et al.*, 1994) and can clearly not be used for comparison here. Data obtained by Eisemann *et al.*,

1997 was not expressed in standard units and the low dose of insulin infused (up to 5.33 mU/kg×min) did not seem to attain the maximum response.

Although a value similar to Figure 1 for sensitivity of fasting glucose metabolism in cattle (66 μU/mL) occurred, it should be considered that this value was obtained from data using the entire clamp period i.e. not SSGIR (Eisemann *et al.*, 1994). A decrease in the sensitivity of the whole-body glucose metabolism response to insulin at 100 to 111 μU/mL has been reported, but it should be kept in mind that hourly feeding of wethers could have distorted the basal, fasting metabolic responses and the reduction could have been the result of some inconsistencies in the experimental protocol (Janes *et al.*, 1985). A value for sensitivity as low as 143 to 258 μU/mL in fed sheep at various stages of the growth cycle was also compromised poor application of the hyperinsulinaemic euglycaemic clamp protocol (Sano *et al.*, 1996).

In Figure 3, the rate of endogenous hepatic glucose production of the human *vs.* ovine species is compared. Even at insulin concentration in plasma greater than 10 000 μU/mL, endogenous glucose production of the ruminant animal was not completely suppressed (Weekes *et al.*, 1983), compared to complete inhibition at 11 μU/mL in man (Rizza *et al.*, 1981). From the basal levels of 2.0 mg/kg×min in man (Rizza *et al.*, 1981) and 1.6 mg/kg×min in sheep (Weekes *et al.*, 1983), half-maximal inhibition was reached at an insulin concentration of 29 μU/mL in man (Rizza *et al.*, 1981) and only 303 μU/mL in sheep (Weekes *et al.*, 1983).

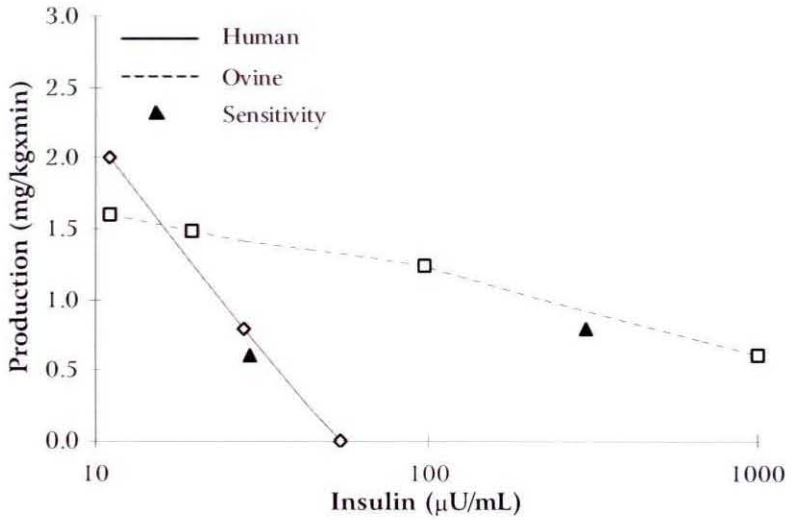

Figure 3. Dose-response curve for glucose production in humans *vs.* sheep (adapted from Rizza *et al.*, 1981 and Weekes *et al.*, 1983)

In Figure 4, adapted from Rizza *et al.*, 1981 and Weekes *et al.*, 1983, the differences in response of peripheral glucose utilization between humans and sheep are summarized. The rate of glucose utilization increased steadily with a rise in insulin concentration of plasma up to a maximum of 9.6 mg/kg×min in man, at insulin concentrations above 679 μU/mL (Rizza *et al.*, 1981). In sheep whole-body glucose utilization only increased to 4.4 mg/kg×min, at insulin concentrations of approximately 1 000 μU/mL plasma, with a much lower responsiveness of glucose to insulin (Weekes *et al.*, 1983). Half-maximal glucose utilization was achieved at 55 μU/mL insulin in man (Rizza *et al.*, 1981), while half-maximal glucose utilization in sheep already occurred at 15 μU/mL (Weekes *et al.*, 1983).

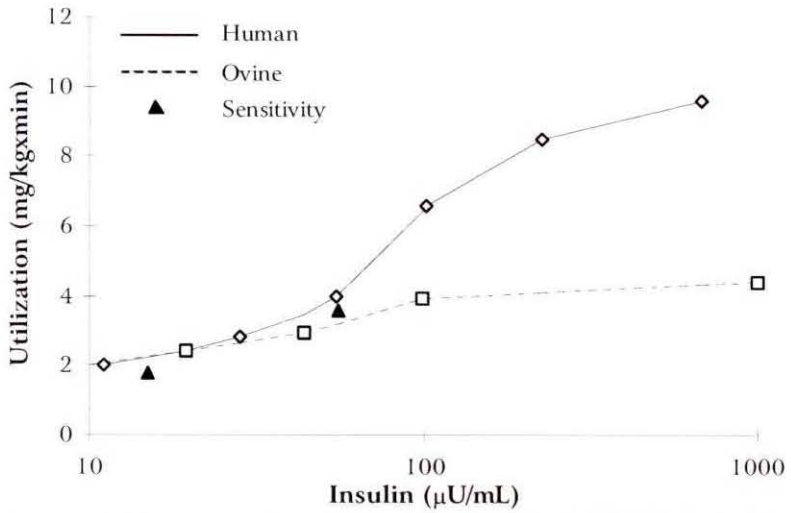

Figure 4. Dose-response curve for glucose utilization in humans *vs.* sheep (adapted from Rizza *et al.*, 1981 and Weekes *et al.*, 1983)

Whereas hepatic glucose production is more sensitive than peripheral tissues to the effects of insulin in man (Rizza *et al.*, 1981), peripheral tissues are more sensitive to the effects of insulin in sheep than the liver (Weekes *et al.*, 1983). The relative insensitivity of the ruminant liver to insulin inhibition of glucose output is understandable, as the supply of glucose in the ruminant animal is almost entirely dependent on gluconeogenesis and should not be compromised. Neither the maximal responsiveness nor the sensitivity of hepatic glucose production was affected during pregnancy in ewes, where altered glucose metabolic responses occurred through modulation of insulin responses in peripheral insulin-dependent tissues (Petterson *et al.*, 1993). The greater tissue sensitivity in the ovine could be a result of the low insulinaemia, but these values are difficult to interpret because insulin-induced utilization cannot always be accurately separated from insulin-independent glucose utilization (Weekes *et al.*, 1983).

1.2 Other notable effects

Care should be taken when interpreting the results of metabolic tests performed in animals where variation in feeding, feeding level, ambient temperature, season, mineral status, age or adiposity occurred, or in animals with ketosis, acidosis, or during disease states. In addition to variations between species (and breeds), experimental protocols, nutritional factors, the environment and metabolic status of animals, the method of challenge delivery can also affect the metabolism of the animal. While the portal glucagon concentration, systemic glucose concentration, hepatic glucose load, systemic to portal glucose concentration gradient, peripheral glucose uptake and peripheral insulin concentration were similar, hepatic glucose uptake was enhanced, hepatic glycogen synthesis was significantly greater with a much greater proportion of blood glucose uptake directed to glycogen synthesis (80%) when insulin was administered in the portal vein of the liver instead of peripherally (60%) under conditions that mimicked the postprandial phase in dogs (Satake *et al.*, 2002).

1.2.1 Feeding and interaction with physiological state

Feeding a poor quality feed (grass-based) to wethers did not affect circulating concentration or basal metabolic clearance rate of either glucose or insulin, or the SSGIR

during hyperinsulinaemic euglycaemic clamps, but decreased the metabolic clearance rate of glucose and the sensitivity of the response to insulin compared to a maize-based diet (Janes *et al.*, 1985). A similar protocol of nutritional limitation in growing steers decreased *in vitro* glucose oxidation to CO_2 and lactate (Rhoades *et al.*, 2007). The lipolytic responses to adrenergic signals are greatly dependant on and modified by the energy balance of animals, where a clear response peak occurred when dry nonpregnant cows were in a negative (–14.8 MJ ME/d) energy balance, compared to a very mild more transient response when in a positive (+34.5 MJ ME/d) energy balance (Ferlay *et al.*, 1996).

In ewes, pregnancy was characterized by increased basal endogenous glucose production, glucose metabolic clearance rate and insulin-independent glucose utilization, with reduced sensitivity of whole-body glucose utilization to insulin coupled to an increase in glucose metabolic clearance rate (Petterson *et al.*, 1993). There was a reduction in the responsiveness of insulin-dependent glucose utilization and a tendency ($P<0.10$) for decreased sensitivity of this response to insulin during pregnancy, while basal glycaemia and insulinaemia were unaffected (Petterson *et al.*, 1993). Undernutrition decreased basal endogenous glucose appearance, metabolic clearance rate and insulin-independent utilization in the face of unchanged insulin or glucose concentration in dry ewes (Petterson *et al.*, 1993), although a lack of effect on basal responses has also been reported in ewes (Metcalf & Weekes, 1990). Whereas insulin-induced whole-body glucose metabolism, glucose metabolic clearance rate and glucose utilization was not affected by nutritional state, the responsiveness of endogenous glucose production was decreased (Petterson *et al.*, 1993) and decreased the sensitivity of glucose clearance in response to insulin (Metcalf & Weekes, 1990).

Period of the feeding cycle affected the GIR during hyperglycaemic clamp up to 2 to 4 hours after feeding in sheep, but did not affect the results of hyperinsulinaemic euglycaemic clamps, although steady-state conditions were not achieved in these experiments (Sano *et al.*, 1990). Insulin and glucose responses to intravenous glucose and insulin challenges were altered in the preprandial *vs.* 3-hour postprandial period in milk replacer-fed dairy calves (Hostettler-Allen *et al.*, 1994). Glucose half-life was increased in the postprandial period, where the insulin peak response was delayed following glucose

injection and the glucose concentration response blunted following insulin injection (Hostettler-Allen *et al.*, 1994).

1.2.2 Body weight, adiposity and stage of the growth phase

A lean BCS in late pregnant ewes resulted in a tendency toward a 20.6% reduction in insulin concentration ($P<0.10$) and resulted in reduced insulin responses to glucose challenge that also affected the distribution of glucose to the gravid uterus, while glucose tolerance remained unaffected (McNeill *et al.*, 1997).

Increasing age was associated with changes in the hormone and metabolite concentrations, with altered metabolic responses like a 39.0% reduction in glucose clearance rate in dairy calves between 2 weeks to 8 weeks of age as normal metabolic responses were established (Depew *et al.*, 1998). The basal arterial glucose concentration was approximately 68 mg/dL in steers weighing both 285 kg (less than 8 months of age) and 490 kg (more than 15 months old), whereas the results of hyperinsulinaemic euglycaemic clamps was very different between groups (Eisemann *et al.*, 1997). While glucose concentrations were similar, younger steers had an arterial insulin concentration of only 22 μU/mL, which was 43 μU/mL in older steers (Eisemann *et al.*, 1997). This was due to apparently greater insulin secretion and decreased hepatic insulin removal, although both values only tended to be different with no indication of the actual level of significance (Eisemann *et al.*, 1997). At 5 months of age the ovine β cell response to exogenous glucose or the mean plasma insulin increment (MPII) was decreased compared to adult sheep (–60.5%) and 9 month old lambs (–75.4%), although care should be taken with these data, as feed was only withdrawn 2 hours before the clamps and hyperglycaemia only reached after 50 minutes (Sano *et al.*, 1996). The whole-body glucose response to hyperinsulinaemic euglycaemic clamp of young, leaner steers *vs.* older steers (with a 29.1% greater fat percentage in the hindquarters) is compared in Figure 6. The authors did not use the standard units to represent glucose data or insulin infusion rates and it did not seem that the maximum response had been reached at insulin infusion of 320 mU/kg×h (only 5.3 mU/kg×min), although these data were adapted and used in the figure below (Eisemann *et al.*, 1997). The net responsiveness of older steers was greater (932 mmol/h) than control (653 mmol/h), but expressed per unit body weight the older

steers had a lower responsiveness of SSGIR estimated at 5.72 mg/kg×min compared to 7.13 mg/kg×min (Eisemann *et al.*, 1997). The sensitivity of the glucose response of older steers was 237 µU/mL compared to 113 µU/mL in younger steers (Eisemann *et al.*, 1997), where the increased sensitivity of the glucose response can be a result of the lower insulin concentrations of younger steers.

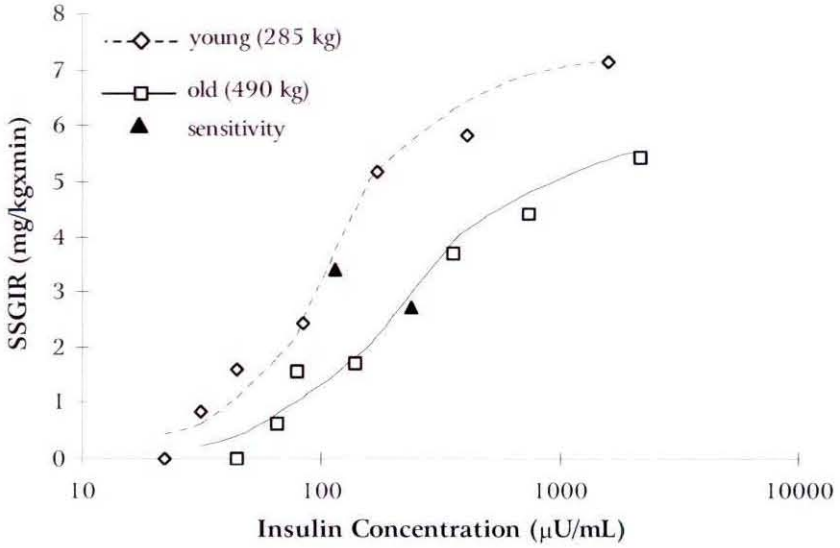

Figure 5. Dose-response curve for SSGIR in younger *vs.* older steers (Eisemann *et al.*, 1997)

1.2.3 Temperature

The basal insulin concentrations of 5.7 µU/mL (Weekes *et al.*, 1983) and nonfasting concentrations of 10.5 µU/mL (Achmadi *et al.*, 2001) were unaffected by exposure to 0°C in rams. The fasting glucose concentration was increased by 14.9%, from 69.6 mg/dL to 80.0 mg/dL in one experiment (Weekes *et al.*, 1983), while the nonfasting glucose concentration remained unaffected at 52 mg/dL by cold in (Achmadi *et al.*, 2001). There was a similar increase in glucose concentration (>+15%) in response to cold when sheep were fed either a medium energy or high-energy diet (Sano *et al.*, 2007). Conversely metabolism was adjusted in a hot environment, where glucagon responses to nutrients like glucose, arginine and butyrate were enhanced and baseline concentrations of non-esterified fatty acids (NEFA) and glucose were decreased in heifers exposed to a hot environment (Itoh *et al.*, 1998).

The responses to cold included a 19.3% increase in the metabolic clearance rate of glucose, a 37.9% increase in the basal glucose irreversible loss rate (Weekes *et al.*, 1983) and a 30 to 60% increase in glucose turnover rate (Sano *et al.*, 2007). During hyperinsulinaemic euglycaemic clamp the SSGIR was increased by a cold environment in sheep, which was coupled to a decrease in the responsiveness of body tissues without altered sensitivity of the response (Weekes *et al.*, 1983, Achmadi *et al.*, 2001). These results are illustrated by data collected from (Achmadi *et al.*, 2001), although it should be considered that the entire last hour was used to estimate SSGIR and Figure 5 adapted from the data does not represent true steady-state conditions. Both hepatic glucose production and peripheral glucose utilization were increased, while the sensitivity of glucose metabolic clearance and hepatic glucose appearance remained unaffected by exposure of rams to 0 °C (Weekes *et al.*, 1983).

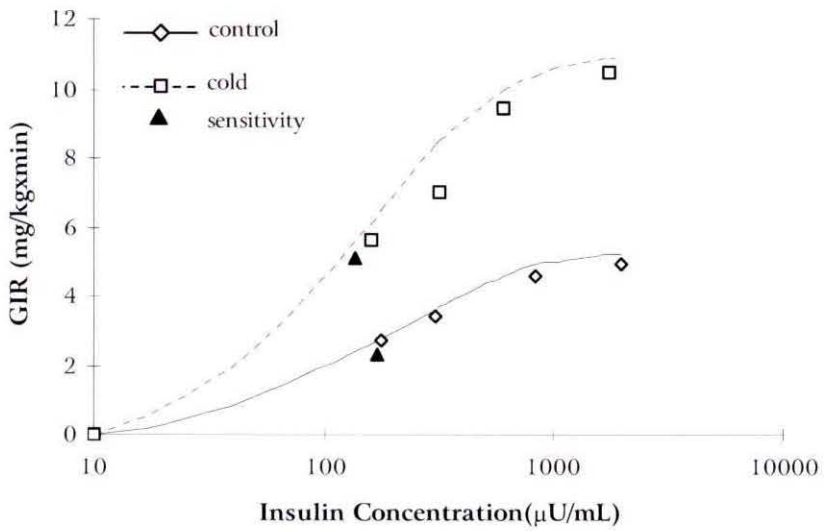

Figure 6. The effect of cold exposure on GIR (Achmadi *et al.*, 2001)

In hyperglycaemic clamps cold exposure did not affect the plateau glucose or insulin concentrations of sheep, while SSGIR was increased by 64.5% (Weekes *et al.*, 1983). This was associated with a 46.3% greater appearance of glucose and 36.7% greater glucose utilization, while the metabolic clearance rate of glucose was increased by 36.4% in rams exposed to an environmental temperature of 0 °C (Weekes *et al.*, 1983). Clearly the results of metabolic tests would then be affected by variation in the environmental temperature and care should be taken when interpreting results where the data for the control and experimental groups were collected in different seasons, like (Eisemann *et al.*, 1997).

1.2.4 Acidosis and ketosis

The acid-base status of cows can affect the results of metabolic tests and a 4.4% reduction in euglycaemia was observed in dry, nonlactating Jersey cows where mild acidosis (blood pH 7.32) was induced (Bigner *et al.*, 1996). Acidosis in ruminant animals can significantly decrease insulin secretion and in dry, nonpregnant cows the baseline insulin concentration was decreased by 33.7% with mild acidosis, compared to a normally slightly alkalotic condition (Bigner *et al.*, 1996). Mild acidosis (blood pH 7.32) not only decreased the basal circulating glucose and insulin concentrations, but also decreased the rate of insulin secretion following glucose challenge (–22.9%), with a greater increase in glucose concentration (+7.2%) in cows fed a high anion diet to induce acidosis (Bigner *et al.*, 1996).

In early lactation Holstein cows the glucose concentration in serum was 50.1 mg/dL for non-ketotic cows, *vs.* 38.0 mg/dL in cows presenting clinical signs of ketosis, although no statistical comparison was made between values (Sakai *et al.*, 1996). In high-production Holstein cows within one month postpartum, the insulin concentration of normal cows was 14.6 μU/mL, compared to only 8.6 μU/mL in cows suffering from clinical ketosis, although no statistical comparison of data was made (Sakai *et al.*, 1996). In cows suffering from clinical ketosis, glucose challenge in early lactation resulted in what seemed a more pronounced peak glucose concentration coupled to a less pronounced peak insulin concentration, although no statistical comparison was drawn between these values (Sakai *et al.*, 1996). However, these authors reported a significant decrease in the rate at which glucose concentration declined, while the decrease in insulin clearance from the circulation reported also seemed ambiguous (Sakai *et al.*, 1996), because the data reported yield an extremely similar slope of fall when corrected for concentration differences between the groups. These results suggest that the efficacy of glucose dosing in treating ketosis is limited by decreased insulin secretion in ruminant animals suffering from induced acidosis (Bigner *et al.*, 1996) or dairy ketosis (Sakai *et al.*, 1996).

1.2.5 Other

Although the magnesium content of the diet did not affect the basal glucose or insulin concentration in either a thermoneutral or cold environment, a significant environment-diet interaction was observed for the response of glucose metabolism to insulin administration (Achmadi *et al.*, 2001). A low magnesium diet (high in potassium) decreased the greater GIR of sheep exposed to cold environmental temperatures by 38.5% and the reduced adaptive response to cold occurred without any change in sensitivity (Achmadi *et al.*, 2001). Therefore the magnesium content of the diet, especially in cold environments can affect the results of metabolic tests. Chromium supplementation can affect the results of metabolic tests in growing cattle (Bunting *et al.*, 1994), during pregnancy and lactation (Subiyatno *et al.*, 1996). Results have been inconsistent (Depew *et al.*, 1998) with variable effects depending on physiological state (Hayirli *et al.*, 2001), although results should be interpreted with care when large changes in intake and energy balance are induced by supplementation.

Disease can affect the normal metabolic responses with notable examples of cystic ovarian disease and displaced abomasum. In lactating high-production dairy cows suffering from cystic ovarian disease the pancreas was resistant to stimulus by glucose, with reductions of approximately 30% in insulin responses to intravenous glucose challenge with no change in glucose clearance (Opsomer *et al.*, 1999). Displaced abomasum in dairy cows was associated with impaired glucose tolerance (Holtenius & Traven, 1990), where decreased intake, milk production, insulin and glucose concentrations were observed along with increased NEFA and β-hydroxybutyrate in early lactation dairy cows (Van Winden *et al.*, 2003).

2. Somatotropin administration in lactation

Somatotropin has several effects in addition to the classic growth effect ascribed to "growth hormone", with more than 450 different effects in 84 cell types ascribed to the somatotropin receptor (Waters *et al.*, 1999). These include the direct metabolic effects of somatotropin in various tissues and the indirect effects on tissues like the mammary gland that seemed to be mediated by IGF-I and the IGFBPs in the IGF system (McGuire *et al.*,

1995a). Much of the effects of somatotropin have been inferred from research conducted by exogenous somatotropin administration, particularly during growth and lactation. Somatotropin receptors have been identified in many tissues, but not the mammary gland (Gluckman *et al.*, 1987) and galactopoietic effects are considered indirect.

2.1 Somatotropin concentration

Administration of slow-release recombinant bST did not affect the periodicity of circulating somatotropin (frequency or duration of peaks), but increased the amplitude of peaks in somatotropin episodic secretion, resulting in a several-fold increase in mean somatotropin concentration (Cisse *et al.*, 1991). Serum somatotropin concentration peaked at almost 5.0 to 5.9-fold endogenous bovine somatotropin (bST) concentration, approximately 2 to 3 hours after 51.5 IU/d subcutaneous pituitary-derived bST injection in lactating cows (Peel *et al.*, 1981, Peel *et al.*, 1982). This peak response was dose-dependent and on both days 1 and 10 of treatment, where 5 IU and 10 IU failed to affect plasma somatotropin concentration, with increases of +188% at 25 IU, +290% at 50 IU and +502% at 100 IU (Eppard *et al.*, 1985b). Although the administration of pituitary-derived bST at both 36 days and 127 days postpartum lead to an increase of somatotropin concentration to approximately 12 ng/mL, this constituted increases of +233.3% at peak and more than 500% in midlactation, due to differences in endogenous somatotropin concentrations (McDowell *et al.*, 1987b). Daily subcutaneous administration was required because circulating somatotropin concentrations remained highly elevated for several hours, but were steadily declining toward control by 24 hours after treatment (Pocius & Herbein, 1986, McDowell *et al.*, 1987a, McDowell *et al.*, 1987b), although it can remain slightly above control for as much as 5 days (Peel *et al.*, 1981). In ewes, subcutaneous treatment with 0.1 mg pituitary-derived bST per kg body weight (1.2 IU/mg) increased the plasma somatotropin concentration by 111.5% (McDowell *et al.*, 1987c). Administration of recombinant bST (sometribove) over 4 lactations continued to sustain an elevated serum somatotropin concentration of 389.5% above control and the sustained production response and lack of competitive binding suggests that antibody production against recombinant bST was not present (Adriaens *et al.*, 1992).

2.2 Production responses

2.2.1 Milk production

Treatment with 5 IU/d to 10 IU/d pituitary-derived bST failed to increase milk production, while the increase was **dose-dependent** between 25 IU, 50 IU and 100 IU/d (Eppard *et al.*, 1985b). However, there was no significant advantage to a dose of 100 IU pituitary-derived bST per day (+31.8%) *vs.* a dose of 50 IU (+28.5%) when high-production cows were treated at 192 days postpartum (Eppard *et al.*, 1985b). The effect on fat-corrected milk yield was similarly dose-dependent for recombinant bST between 23.3% (18.9 IU/d) up to 41.2% (56.7 IU/d), over a 16-week treatment period in high-producing cows (Bauman *et al.*, 1985a). Recombinant bST was a more effective galactopoietic treatment leading to a 36.2% increase in the fat-corrected milk yield (37.8 IU/d for 104 days from week 13 postpartum) in high yielding dairy cows compared to only a trend toward a 16.5% increase ($P < 0.10$) using pituitary-derived bST (Bauman *et al.*, 1985a). The increase in milk production in response to recombinant bST (40 mg sometribove injected daily) was as high as 41.2% in cows treated for 12 days at 190 days postpartum (Sechen *et al.*, 1990). The effects of exogenous bST treatments on milk production were short-lived and the increase in milk production observed after 10 days of subcutaneous pituitary-derived bST injections returned to the baseline level within 3 days of the end of bST treatment (Peel *et al.*, 1982), with similar results following recombinant bST daily administration (Sechen *et al.*, 1990).

Ten days of treatment with 50 IU/d pituitary-derived bST very early in lactation when cows were in a negative energy balance only tended ($P < 0.09$) to increase milk production from 36.2 kg/d by 6.1% (Richard *et al.*, 1985). Six days of treatment with 0.1 IU/kg body weight pituitary-derived bST in dairy cows at peak (36 days postpartum) increased milk production by only 6.0%, which was related to an 8.5% increase in the fat-corrected milk yield (McDowell *et al.*, 1987b). These responses were reduced compared to similar applications later in lactation. Treatment with 50 IU/d pituitary-derived bST at 60 days postpartum when cows were in an almost zero energy balance tended ($P < 0.08$) to increase milk production from 34.6 kg by 11.8% (Richard *et al.*, 1985). Six days of 0.1 IU/kg pituitary-derived bST in midlactation (127 days postpartum), increased milk yield by 14.0% and fat-corrected milk yield by 19.4% (McDowell *et al.*, 1987b). The apparent

lack of response at 20 days *vs.* 60 days postpartum (Richard *et al.*, 1985) or 36 days *vs.* 127 days postpartum (McDowell *et al.*, 1987b) could also be ascribed to higher endogenous somatotropin in early lactation, rather than differences in energy balance. Even when cows producing 34.4 kg milk per day (74 days postpartum) were in a significantly negative energy balance (–11.0 Mcal/d), 51.5 IU pituitary-derived bST administration for 10 days increased milk production by 9.5% (Peel *et al.*, 1981). Daily injection of sheep with 0.1 mg pituitary-derived bST per kg body weight (1.2 IU/mg) for 4 days, at 50 days postpartum, increased milk yield by 12.4% (McDowell *et al.*, 1987c).

Treatment with 39 IU/d pituitary-derived bST for 22 weeks increased overall milk production by 17.7% in pasture-fed twin cows, which returned to baseline levels within 7 days after pituitary-derived bST treatment (Peel *et al.*, 1985). However, the 33.7% increase in fat-corrected milk yield at 4 weeks was reduced to a 26.1% increase by week 8, with no difference evident by 22 weeks postpartum, i.e. **persistency** of milk production was not enhanced by pituitary-derived bST (Peel *et al.*, 1985). Similarly long-term (104-day) administration of pituitary-derived bST failed to elicit a significant increase in milk production (+16.5%, $P < 0.10$), because milk production rapidly declined toward control levels after an initial increase in milk production (Bauman *et al.*, 1985a). However, administration of recombinant bST maintained elevated milk production throughout the treatment period, changing the shape of the lactation curve, enhancing the persistency of milk production (Bauman *et al.*, 1985a). The response to recombinant bST (500 mg sometribove) was sustained over several lactations with a milk production 24.4% greater than control still evident at approximately 120 days postpartum in the fourth lactation of treatment from 60 days postpartum, although this did not constitute an increase in the fat-corrected milk yield (Adriaens *et al.*, 1992). Administration of slow-release subcutaneous recombinant bST (160 mg) over the entire lactation of dairy ewes increased 6% fat-corrected milk yield by 22.1%, from just after weaning up to near the end of lactation (Brozos *et al.*, 1998). Although the persistency of milk production was not increased by long-term administration of recombinant bST in dairy ewes, it should be considered that these ewes were not allowed to adapt feed intake to treatment (Brozos *et al.*, 1998).

Poor nutrition modulated the responses of the somatotropic axis (Gluckman *et al.*, 1987) and prevented many of the normal responses in the somatotropic axis (McGuire *et al.*, 1995a, Renaville *et al.*, 2002). Treatment of early lactation dairy cows on a control (100% of requirements) diet increased milk production to a greater extent than a nutrient concentrated diet alone, with the greatest response observed in cows receiving both the high protein and energy diet plus exogenous bST (Austin *et al.*, 1991). Restricting either net energy or crude protein intake to 80% of the calculated requirements decreased milk production and its response to 4 day recombinant bST treatment in a similar manner, while restriction of both components did not prevent the response to bST of the reduced milk production of these cows (McGuire *et al.*, 1992). This was also demonstrated by the lack of effect of 500 mg recombinant bST treatment of cows fed a low-protein concentrate and turned out to pasture at 24 weeks postpartum, 15 weeks into treatment (Chilliard *et al.*, 1991). In this experiment using multiparous Holstein cows, milk yield tended to increase ($P < 0.10$) in response to slow-release recombinant bST in the first 9 weeks of administration by only 3.2 kg/d, with no effect in the following 19 weeks of treatment (Chilliard *et al.*, 1991). Not only was the absolute gain in milk yield increased as the nutrient concentration of the diet for crude protein and metabolizable energy (ME) increased, but the percentage increase in milk yield also exhibited stepwise increase (Newbold *et al.*, 1997).

2.2.2 Milk fat

Milk fat yield was increased in a dose-dependent manner by 25 IU (+15.5%), 50 IU (+35.1%) and 100 IU (+46.4%) pituitary-derived bST treatment for 10 days, where cows were in a positive energy balance at the start of treatments (Eppard *et al.*, 1985a). The milk fat percentage was not affected by pituitary-derived bST treatment of cows at 192 days postpartum, where treatments did not cause appreciable changes in the positive energy balance (Eppard *et al.*, 1985b). A 104 day treatment with 18.9 IU up to 56.7 IU/d recombinant bST, which increased milk production by between 23.3% to 41.2% did not affect milk fat percentage, where treatments were initiated after peak when cows were in a positive energy balance (Bauman *et al.*, 1985a). Similarly chronic treatment with pituitary-derived bST, which only tended to increase fat-corrected milk production, did not affect the milk fat percentage of cows where the positive energy balance was not significantly

altered (Bauman *et al.*, 1985a). A 12.4% increase in milk production induced by subcutaneous injection of pituitary-derived bST in lactating sheep was accompanied by a 10.8% increase in milk fat content (McDowell *et al.*, 1987c). However, in recombinant bST treated ewes fed a fixed amount of feed daily, no change in the milk fat content occurred over the entire lactation period (Brozos *et al.*, 1998). Changes in milk fat yield were associated with small increases in the milk fat percentage with 50 IU/d pituitary-derived bST administration (+5.4%), which were statistically similar to the 9.7% increase observed at 100 IU/d where energy balance was decreased to values near zero to slightly negative (Eppard *et al.*, 1985b).

When cows in a positive energy balance (+5.5 Mcal/d) were exposed to daily injections of recombinant bST for 12 days that lead to a negative energy balance (–5.4 Mcal/d), the large decline in energy balance was associated with a significant increase in the milk fat percentage (+25.8%) in midlactation (Sechen *et al.*, 1990). When cows in a significantly negative energy balance (–5.7 Mcal/d) were exposed to pituitary-derived bST in early lactation, the increase in milk fat yield of 25.3% was coupled with a 20.3% increase in milk fat percentage (Richard *et al.*, 1985). A very similar increase of 24.8% in milk fat yield was observed later in lactation when cows were in a slightly positive energy balance later in lactation, but was not associated with an effect in milk fat percentage (Richard *et al.*, 1985). An increase in milk fat percentage of up to 28.9% has been reported in midlactation cows treated with pituitary-derived bST for 14 days (Bitman *et al.*, 1984), where the already-negative energy balance (– 1.1 Mcal/d) was decreased to –9.8 Mcal/d by treatment (Eppard *et al.*, 1985b). Even with a very negative energy balance (–11.0 Mcal/d digestible energy balance in high-production cows near peak, the milk fat yield was increased by 22.7%, associated with a 12.9% increase in milk fat percentage (Peel *et al.*, 1981).

Milk **fat composition** was altered by treatment with bST, an effect that is dependent on the energy balance of treated cows (Bauman *et al.*, 1988), where mobilized fatty acids become a more important component of milk lipid (Bitman *et al.*, 1984). Ten days of treatment with 5 IU and 10 IU/d pituitary-derived bST generally failed to affect the milk fat composition of high-producing dairy cows (Eppard *et al.*, 1985a), where no significant

effects on production responses were observed (Eppard *et al.*, 1985b). In well-fed Holstein cows treated with exogenous bST in early lactation no change in the percentage or composition of milk fat was observed for individual fatty acids or different classes of fatty acids (Austin *et al.*, 1991). The short and medium-chain fatty acid content of milk was decreased by 10.7% by 14 days of pituitary-derived bST treatment, because the increase in *de novo* synthesis of fatty acids did not match the increase in milk fat yield (Bitman *et al.*, 1984). The C14:0 production (as a percentage of total fatty acids) was decreased by 5.9% (50 IU/d) and by 12.5% (100 IU/d) after 10 days of pituitary-derived bST treatment, while C14:1 was decreased by 15.0% by 100 IU/d (Eppard *et al.*, 1985a). The reduction in the content of shorter-chain fatty acids in milk fat was very closely matched by an increase in the fatty acid content of preformed fatty acids, mobilized from body reserves where a significant reduction in energy balance was evident (Bitman *et al.*, 1984). The C16:1 proportion in milk fatty acids was increased in a dose-dependent manner (Eppard *et al.*, 1985a) by 50 IU (+11.1%) and 100 IU (+25.9%) pituitary-derived bST treatment for 10 days, where C 18:1 was also increased by 50 IU (+9.4%) and 100 IU (+18.9%) when energy balance was greatly decreased (Eppard *et al.*, 1985b). Similarly in midlactation cows, exposure to recombinant bST increased C16:1 (+30.8%) and C18:1 (+14.4%) abundance in the absence of altered milk fat percentage, while the content of short, medium and other long-chain fatty acids were not affected by treatment (Beswick & Kennelly, 2000). A 26.6% increase in C18:1 was also observed in cows experiencing a significant increase in milk fat percentage in midlactation (Bitman *et al.*, 1984). However, the total long-chain fatty acids were not consistently affected by 10 days of pituitary-derived bST, with small non-significant decreases in some of the long-chain fatty acids (Eppard *et al.*, 1985a). Two weeks of exposure to pituitary-derived bST decreased the structural lipids of milk, with a decrease of 20.6% in cholesterol and 28.9% in phospholipid, while the triacylglycerol and diacylglycerol percentage remained unaffected, with greater total percentage milk fat (Bitman *et al.*, 1984).

2.2.3 Milk lactose

Milk lactose yield of dairy cows and ewes generally reflects the changes in milk production without an effect on milk lactose content, because of its important osmotic function in milk. A small but significant decrease (–4.5% of control) in milk lactose percentage was

observed with 0.1 IU/kg body weight pituitary-derived bST for 6 days at peak, because the increase in milk lactose yield was not significant (McDowell *et al.*, 1987b). Milk lactose yield was increased by as much as 20.6% following 10 days of 51.5 IU/d pituitary-derived bST subcutaneous injection, without any change in the milk lactose percentage (Peel *et al.*, 1982).

The yield of the milk protein α-lactalbumin (an important component of the *lactose synthase* complex) was unaffected by 10 days of 5 IU and 10 IU/d pituitary-derived bST treatment and increased in a dose-dependent manner by 25 IU (+35.0%), 50 IU (+46.1%) and 100 IU (+73.4%) per day (Eppard *et al.*, 1985a). At 100 IU/d pituitary-derived bST the α-lactalbumin concentration in milk was increased by 32.3%, while 50 IU and 100 IU/d increased the lactalbumin proportion of total milk protein (Eppard *et al.*, 1985a), even while the milk protein percentage was decreasing (Eppard *et al.*, 1985b).

2.2.4 Milk protein

Milk protein yield was generally increased in a similar manner to the milk yield response with no difference in the response to pituitary-derived bST *vs.* recombinant bST when cows remained in a zero or positive nitrogen balance (Eppard *et al.*, 1985b, Sechen *et al.*, 1989). Intramuscular administration of 37.8 IU/d and various doses of recombinant bST (18.9 IU/d, 37.8 IU/d and 56.7 IU/d) for 104 days from 4 weeks after peak yield did not affect milk protein content (Bauman *et al.*, 1985a). The protein yield of cows treated with 25 IU, 50 IU and 100 IU/d pituitary-derived bST for 10 days increased in a dose-dependent manner, between 15.6% up to 26.7% (Eppard *et al.*, 1985a), where 25 IU/d failed to affect the milk protein percentage when the nitrogen balance was unaffected (Eppard *et al.*, 1985b). Higher doses of pituitary-derived bST for 10 days decreased the nitrogen balance by 4 g/d to 6 g/d and also decreased the milk protein percentage by 4.1% to 4.7% (Eppard *et al.*, 1985b). The milk protein yield only tended to increase after pituitary-derived bST treatment of midlactation cows in a significantly negative nitrogen balance (–21g/d), which lead to a decrease in milk protein percentage of 6.3% of control values, coupled with an increase (+10.2%) in milk nitrogen excretion (Tyrrell *et al.*, 1988). When the protein requirement was not met by the total mixed ration, 12 days of subcutaneous recombinant bST treatment tended to decrease the protein content of milk

by 8.0% (significance level not available), where the increase in milk protein yield failed to match the rise in milk production (Sechen *et al.*, 1990). Short-term exposure to recombinant bST that failed to increase protein yield lead to a tendency ($P < 0.07$) toward a 1.8% decrease in milk protein percentage (Molento *et al.*, 2002). This was associated with a small but significant increase in the casein percentage (+4.1%) in early lactation cows, coupled to a 10.8% reduction in milk urea concentration (Molento *et al.*, 2002). When the response of milk protein yield paralleled the increase in milk yield no change in the milk urea-nitrogen occurred in midlactation cows treated with slow-release recombinant bST (Velez & Donkin, 2004). Although the reduction in near-zero nitrogen balance to slightly negative values did not reach statistical significance, pituitary-derived bST administration decreased the milk protein percentage of early lactation dairy cows by 4.6% (Peel *et al.*, 1981).

2.2.5 Milk energy and the efficiency of milk production

Milk energy secretion (Mcal/d) exhibited a dose-dependent increase in short-term studies, where intake was unaffected by exogenous bST, although changes in milk energy are highly variable, even when results of the same pituitary-derived bST dose within the same laboratory were compared (Eppard *et al.*, 1985b). Because milk production responses to somatotropin present without any change in intake, the gross efficiency of milk production is often increased during short-term periods of bST administration. However, it must be considered that the balance of nutrients at tissue level can have a large impact on the values for gross efficiency (kg product ÷ kg dry matter intake) obtained from experimental animals (Veerkamp & Emmans, 1995).

Daily administration of 5 IU and 10 IU pituitary-derived bST failed to affect circulating bST concentration or milk production responses (including milk energy yield), but still increased the efficiency of milk production by 8.3% and 9.9% respectively (Eppard *et al.*, 1985b). An intermediate response was observed at 25 IU/d where milk energy was increased by 16.1% (+3.1 Mcal/d) leading to a 14.9% increase in efficiency, although many of the milk production responses remained similar to control (Eppard *et al.*, 1985b). Treatment with 50 IU and 100 IU/d pituitary-derived bST increased milk energy yield in a similar fashion by approximately 30%, but the efficiency of milk production was

increased by 27.3% by 50 IU/d and by 37.2% during 100 IU/d treatment, in part due to a reduction in feed intake in this group (Eppard *et al.*, 1985b). Daily milk energy output was increased by as much as 54.5% (+9.6 Mcal/d) in midlactation Holstein cows receiving daily injections of recombinant bST (Sechen *et al.*, 1990). The increase in apparent efficiency has been as high as +30.6% in midlactation cows treated with 51.5 IU/d, where milk energy secretion was increased by 24% in the face of a very large increase (+28.9%) in the milk fat percentage and occurred at the expense of a large increase in the proportion of tissue energy loss (Tyrrell *et al.*, 1988).

As early as 20 days postpartum (in cows producing 36.2 kg milk per day), milk energy secretion in high-producing dairy cows was increased by 16.6% (+4.4 Mcal/d) by pituitary-derived bST administered for 10 days, which tended to increase ($P < 0.09$) the efficiency of milk production by 15.7% (Richard *et al.*, 1985). In cows at 60 days postpartum, 10 days of treatment resulted in an 18.9% increase in milk energy (+4.5 Mcal/d), which was associated with a significant increase of efficiency of milk production of 17.7% (Richard *et al.*, 1985). These values are very similar to the increased daily milk energy output of 17.1% (+4.05 Mcal/d) following 10 days of 51.5 IU/d pituitary-derived bST treatment near peak (Peel *et al.*, 1981) and an increase of 17.4% (+3.4 Mcal/d) following treatment at 81 days postpartum (Peel *et al.*, 1982).

Long-term administration of 37.8 IU/d pituitary-derived bST failed to elicit a change in the milk energy output (Mcal/d), as the increase in milk production failed to reach statistical significance because persistency of milk production was not increased (Bauman *et al.*, 1985a), or the intake was matched to production (Peel *et al.*, 1985). The administration of recombinant bST was more effective than pituitary-derived bST. After 104 days of 37.8 IU/d recombinant bST daily milk energy output increased by 36.7%, which related to +7.2 Mcal/d, while pituitary-derived bST failed to elicit a significant response (Bauman *et al.*, 1985a). A comparable increase in milk energy excretion (+7.1 Mcal/d) was attained by daily administration of 100 IU/d pituitary-derived bST for 10 days (Eppard *et al.*, 1985b).

2.2.6 Milk minerals

There was a variable response of the minerals in milk, including calcium, phosphorous, sodium, zinc (Zn), iron, copper and manganese, after 10 days of pituitary-derived bST exposure between 5 IU to 100 IU/d in high-producing dairy cows (Eppard *et al.*, 1985a). The same authors also reported a lack of effect of 104 days of pituitary-derived and recombinant bST administration on the calcium and phosphorous content of milk (Bauman *et al.*, 1985a). The milk magnesium concentration was the only consistent increase following 10 days of 25 IU, 50 IU and 100 IU/d pituitary-derived bST and was increased by as much as 6% by the two greatest doses of exogenous somatotropin (Eppard *et al.*, 1985a).

2.3 Metabolic responses

2.3.1 Glucose, insulin and glucagon concentrations

Care should be taken when interpreting what has been termed a diabetogenic effect of somatotropin administration in lactation (Gluckman *et al.*, 1987) especially of pituitary extracts, because pituitary hormones and vehicle for delivery could potentially falsely alter glucose and insulin homeostasis (Eppard *et al.*, 1985b, Bauman *et al.*, 1988). Furthermore the changes in the interaction between insulin and glucose in response to exogenous bST are normal adaptive responses and not a collapse of the normal functional responses associated with diabetes mellitus (Vicini *et al.*, 1991). Glucose concentration remained unaffected in cattle (Peel *et al.*, 1981, Sechen *et al.*, 1990) and ewes (McDowell *et al.*, 1987c). Similarly both the circulating insulin (Peel *et al.*, 1981, Sechen *et al.*, 1989, Adriaens *et al.*, 1992) and glucagon (Peel *et al.*, 1982, Velez & Donkin, 2004) concentrations were unaffected by varying doses of exogenous somatotropin, at different stages of lactation and growth hormone-releasing hormone failed to affect basal glycaemia or insulinaemia in cows at peak and late lactation (Rose *et al.*, 1996). A relatively large decrease in insulin (–34.5%) was noted in late lactation dairy cows that had been exposed to slow-release recombinant bST for 110 days (Newbold *et al.*, 1997), while a very large increase was observed in late lactation cows (Vicini *et al.*, 1991, McGuire *et al.*, 1992), although this was not associated with a significant (5.0%, $P<0.10$) effect on glycaemia (McGuire *et al.*, 1992). The "diabetogenic" effect of somatotropin therefore does not seem

to include a hyperglycaemic-hyperinsulinaemic condition, but rather an altered state of glucose metabolism in response to insulin during lactation.

There were tendencies ($P < 0.10$) toward small increases in blood glucose concentration of 4.9% at peak and 8.0% in midlactation dairy cows, where an increase in insulin concentration was observed in some cows treated with 51.5 IU/d pituitary-derived bST for 6 days (McDowell *et al.*, 1987b). However, when one considers that only 5 cows were used in this experiment and that the concentrations were obtained from a single sample on day 1 of treatment (McDowell *et al.*, 1987b), the apparently large effect on plasma insulin concentration (+200% in midlactation) should be interpreted with care. After 4 lactations of recombinant bST treatment the plasma glucose concentration of control cows at approximately 120 days postpartum was 74.4mg/dL and 10.3% greater in treatment cows (82.1 mg/dL) with no difference in the serum insulin concentrations between groups (Adriaens *et al.*, 1992). The physiological relevance of this change is debatable in control *vs.* treatment groups that were diverging over several lactations, although it could be a result of treatments establishing a new steady-state in glucose metabolism (Adriaens *et al.*, 1992).

A similar lack of glucose or insulin response was noted in wethers acutely exposed to exogenous somatotropin (Rose & Obara, 1996). In growing steers the plasma glucose concentration was increased by 10.3% and the serum insulin concentration by 114.3% during intramuscular treatment with recombinant bST (Boisclair *et al.*, 1994). An acute increase in glycaemia and insulinaemia, accompanied by a decline in circulating glucagon concentration has been reported in pigs (Wray-Cahen *et al.*, 1993). In these animals the large drain on circulating glucose induced by the mammary gland was absent during growth and glucose was freely available in the monogastric animal. During late lactation and the dry period in Holstein cows, recombinant bST increased serum insulin concentration when adaptive responses would have been mild compared to early lactation, were no insulin response was observed (Vicini *et al.*, 1991). These differences were ascribed to differing availability of glucose during the different physiological stages of cows' production cycle (Vicini *et al.*, 1991) and the insulin response occasionally observed in cows during later lactation could depend on the supply of excess alimentary nutrients

including energy and protein (McGuire *et al.*, 1992). A transient rise in glycaemia and insulinaemia was also reported in mares at maintenance (Buonomo *et al.*, 1996) treated with daily and slow-release preparations of either recombinant bST or recombinant porcine somatotropin (pST). A variable chronic diabetogenic effect of both pituitary-derived and recombinant pST has been reported in growing pigs (Gopinath & Etherton, 1989a) and a rise in glucose concentration that occurs when there is no significant drain from the circulation may directly result in the rise in insulinaemia (Vicini *et al.*, 1991).

2.3.2 Glucose metabolism

The glucose response area under the curve (AUC) from t0 to t30 after **insulin challenge** tended to be decreased by 20.5% ($P < 0.10$), while the rate of glucose decline was decreased by 30.8% by 14 days of pituitary-derived bST treatment in early lactation Holstein cows (Sechen *et al.*, 1989). Similarly recombinant bST administration for 12 days decreased the glucose AUC by 32.4% and the glucose removal rate by 41.4% following very similar insulin responses to insulin challenge in midlactation cows (Sechen *et al.*, 1990). Similar results were evident during growth in pigs treated with pituitary-derived pST (Wray-Cahen *et al.*, 1993) and recombinant pST (Kerber *et al.*, 1998), where the glucose response area and removal rate were decreased in the face of unaltered insulin response AUC.

In **hyperinsulinaemic euglycaemic clamp** that lasted 6 days, total GIR required to maintain glycaemia was 20.8% lower in cows also treated with recombinant bST in early lactation (Molento *et al.*, 2002), indicative of an additional glucose-sparing effect. Glucose responses at peak in Holstein cows remained unaffected by exposure to growth hormone-releasing hormone, likely due to the already-modulated metabolic status of cows this early in lactation (Rose *et al.*, 1996). In late lactation the rise in somatotropin concentration induced by growth hormone-releasing hormone decreased glucose turnover by 16% and induced an 18.3% reduction in the metabolic clearance rate of glucose (Rose *et al.*, 1996). In dairy cows (Rose *et al.*, 1996), wethers (Rose & Obara, 1996) and growing barrows (Wray-Cahen *et al.*, 1993), the SSGIR required to maintain glycaemia was significantly decreased by exogenous somatotropin administration, indicating a decreased utilization of glucose in response to hyperinsulinaemic euglycaemia.

Glucose response to metabolic challenges exhibited variable results. The glucose response to **epinephrine challenge** at 2 months postpartum was unaffected by 50 IU/d subcutaneous pituitary-derived bST injection at 13 days of treatment (Sechen *et al.*, 1989). Similarly the increase in glucose response area failed to reach statistical significance in cows treated with a relatively low dose of pituitary-derived bST in late lactation, but still lead to a 28% increase in milk production (McCutcheon & Bauman, 1986).

The glucose response area, removal rate and the half-life of clearance remained unaffected after **glucose challenge** of cows treated with 50 IU/d pituitary-derived bST at 61 days postpartum (Sechen *et al.*, 1989). Similarly the peak glucose concentration, time to peak and the return to baseline were similar to control in cows exposed to recombinant bST for several lactations (Adriaens *et al.*, 1992). The exposure to exogenous bST for a fortnight did not affect the insulin response to glucose challenge in cows (Sechen *et al.*, 1989). Although the time to peak insulin concentration and total insulin AUC were similar between treatment groups, long-term exposure to recombinant bST for 4 lactations tended to decrease ($P < 0.09$) the peak insulin concentration following glucose challenge by 34.9% (Adriaens *et al.*, 1992). Although the administration of pituitary-derived pST to growing barrows also failed to affect the glucose response area, the half-life of glucose was almost doubled with a larger insulin AUC in response to glucose (Wray-Cahen *et al.*, 1993) and a decrease in glucose clearance in the face of a larger insulin response (Gopinath & Etherton, 1989b).

The results of **glucagon challenge** were unaffected by 12 days of pituitary-derived bST treatment in early lactation cows (Sechen *et al.*, 1989).

Basal hepatic glycogen content was decreased by 24.7% by 8 weeks of recombinant bST treatment (Knapp *et al.*, 1992) and remained unaffected by pituitary-derived bST administration for 11 days in midlactation cows (Pocius & Herbein, 1986). The irreversible loss of glucose was increased by 12.4% by 2 weeks of pituitary-derived bST administration during midlactation, with a similar proportion (67.7%) of this greater loss directed toward lactose synthesis (Bauman *et al.*, 1988). The irreversible loss rate of

glucose was increased by 28.3% on the third day of 0.1 IU/kg pituitary-derived bST treatment at 36 days postpartum, while no change in irreversible loss rate was observed at midlactation (McDowell *et al.*, 1987b). The irreversible loss of glucose correlated (r = 0.77) with the yield of milk lactose, with approximately 67.7% of glucose loss directed toward lactose synthesis in control and pituitary-derived bST treated cows (Bauman *et al.*, 1988). Although daily treatment with pituitary-derived bST did not affect the proportion of glucose used for lactose synthesis, the increase in milk production was mediated by the greater total glucose supply to the mammary gland (at least 1.3 mol/d) and the change in glucose irreversible loss rate correlated strongly (r = 0.82) with the change in lactose yield (Bauman *et al.*, 1988).

Total CO_2 production was not affected by daily pituitary-derived bST treatment in midlactation cows (Tyrrell *et al.*, 1988), with only 4.7% of total CO_2 derived from glucose in high-production dairy cows (Bauman *et al.*, 1988). Although the irreversible loss rate of glucose was increased, pituitary-derived bST induced a sparing effect on glucose by decreasing the proportion glucose oxidized to CO_2 by 29.3% and the contribution of glucose to total CO_2 production (from 4.7% in control to 3.8%) by 19.1% (Bauman *et al.*, 1988).

The arterio-venous plasma glucose difference across the hindlimb was decreased by 88.2% by 3 to 4 days of pituitary-derived bST administration at 11 weeks postpartum (McDowell *et al.*, 1987a) and by 18.4% in growing steers receiving recombinant bST (Boisclair *et al.*, 1994). Although the percentage extraction of glucose in leg muscle seemed greatly decreased, this difference did not reach statistical significance compared to pre-injection levels, but was decreased by 59.2% compared to a week of saline treatment following the treatment week (McDowell *et al.*, 1987a). In growing steers a 22.4% reduction in glucose uptake by the hindlimb was observed, coupled to a net output of lactate despite increased insulinaemia (Boisclair *et al.*, 1994). However, the arterio-venous glucose difference and percentage glucose extraction across mammary tissue was not affected by treatment with bST but considering the increase in blood flow, the net uptake of glucose would still be enhanced (McDowell *et al.*, 1987a). The lactate concentration of whole-blood was not affected after a few days of 0.14 IU/kg body weight pituitary-derived

bST treatment with no significant effects on the arterio-venous difference, or percentage extraction of lactate by skeletal muscle and mammary tissues (McDowell *et al.*, 1987a). Although these authors suggest a significant increase in the output of lactate from the hindlimb, it should be considered that these large increases were non-significant ($P > 0.10$) and only three cows were used in the experiment (McDowell *et al.*, 1987a). In growing steers a significant effect of 11 to 13 days of recombinant bST administration was noted, with an 8.4% increase in the arterial concentration, where the arterio-venous difference became negative resulting in a net release of lactate from the hindlimb, or skeletal muscle in response to exogenous somatotropin (Boisclair *et al.*, 1994).

2.3.3 Lipid metabolism

It is suggested that highly-purified pituitary-derived bST is not lipolytic during lactation and this effect is a result of extract impurity (Eppard *et al.*, 1985b). However several other authors have observed a significant rise in circulating NEFA that could also be an indirect consequence of the negative energy balance (Peel & Bauman, 1987) and lipid mobilizing response to accommodate an increase in milk production in the absence of an increase in intake.

The **NEFA concentration** remained unchanged when low-dose pituitary-derived bST failed to affect the positive energy balance (Eppard *et al.*, 1985b, McCutcheon & Bauman, 1986), or where the reduction in energy balance was not large (Eppard *et al.*, 1985b). A lack of NEFA response to exogenous bST in cows near peak that exhibited a reduction in body weight (McDowell *et al.*, 1987b) could be due to the already-elevated somatotropin and body fat mobilization that were not further enhanced (Bell, 1995). However, an increase in NEFA concentration was generally observed when a negative energy balance was induced by somatotropin administration (Peel *et al.*, 1981, Eppard *et al.*, 1985b, Sechen *et al.*, 1989, Sechen *et al.*, 1990). It seemed that the major effect of somatotropin administration when animals were in a positive energy balance was on the rate of lipogenesis (Lanna *et al.*, 1995), while effects in animals with a ≤ 0 energy balance were aimed at altering the rate of lipolysis (Etherton & Bauman, 1998). The apparent increase in NEFA concentration and lipolysis could be ascribed to the change in responses to

homeostatic signals, rather than direct effects of somatotropin on lipolysis (Etherton & Bauman, 1998).

A small increase in NEFA concentration of 22.8% was reported after exposure to 100 IU/d highly-purified pituitary-derived bST, where the energy balance became slightly negative (Eppard *et al.*, 1985b). The chronic lipolytic response to exogenous somatotropin has been reported as high as +372.0%, where midlactation cows in a positive energy balance (+5.5 Mcal/d) were treated with recombinant bST for 12 days, which induced a significantly negative energy balance of –5.4 Mcal/d (Sechen *et al.*, 1990). The NEFA concentration in plasma was increased by 51.5% in midlactation cows and was coupled to a decrease in the total lipid concentration (–6.5%), with a tendency ($P < 0.10$) toward a 19.1% decrease in the total fatty acid (including phospholipids, cholesterol esters and triacylglycerols) concentration (Bitman *et al.*, 1984). Similarly an increase in NEFA concentration (+98.8%) and NEFA irreversible loss rate (+20.2%) was observed with pituitary-derived bST administration in midlactation, while NEFA concentration and irreversible loss rate remained unaffected at peak (McDowell *et al.*, 1987b). An increase in hepatic lipid content (+38.1%) was observed in cows exposed to subcutaneous bST in early lactation, but was not associated with altered hepatic triacylglycerol, plasma NEFA or plasma β-hydroxybutyrate concentrations (Pershing *et al.*, 2002).

The plasma **glycerol concentration**, which is indicative of the lipolytic response without a confounding effect of re-esterification, was increased by 119.4% in midlactation cows treated with recombinant bST, whereas the NEFA response, confounded by possible reductions in re-esterification, was far more pronounced at +372.0% (Sechen *et al.*, 1990). In dry nonpregnant ewes 6 days of bST treatment increased plasma glycerol by 30.7% (Doris *et al.*, 1996).

The plasma **acetate concentration** was not affected by day 2 of 0.1 IU/kg body weight pituitary-derived bST treatment, with no effect on the irreversible loss rate of acetate (McDowell *et al.*, 1987b). Similarly, acetate concentration in arterial blood, uptake of acetate by the hindlimb (McDowell *et al.*, 1987a, Boisclair *et al.*, 1994) and by the mammary gland was not affected by exogenous bST treatment (McDowell *et al.*, 1987a).

The circulating **β-hydroxybutyrate** concentration was unaffected by short-term exogenous bST exposure of cows at various stages of lactation (Pocius & Herbein, 1986, McDowell *et al.*, 1987a, McDowell *et al.*, 1987b, Pershing *et al.*, 2002) and increased by 13.7% in early lactation Holstein cows exposed to recombinant bST for 2 weeks (Rose *et al.*, 2005). Whole-blood β-hydroxybutyrate concentration and uptake into mammary gland and muscle tissues were also not affected by treatment at 11 weeks postpartum (McDowell *et al.*, 1987a). The arterial β-hydroxybutyrate concentration was decreased by 6.5% by treatment with recombinant bST in growing steers, which was also associated with a decrease in the arterio-venous difference and the net uptake of β-hydroxybutyrate by the hindlimb (Boisclair *et al.*, 1994). There was no effect of 10 days of pituitary-derived bST treatment on the circulating acetoacetate concentration at 120 days postpartum (Pocius & Herbein, 1986).

Treatment with pituitary-derived bST (50 IU/d for 13 days) enhanced the NEFA (or lipolytic-esterification) response to **epinephrine challenge** with a 124.8% increase in the response area in early lactation Holstein cows, where treatment induced a negative energy balance (Sechen *et al.*, 1989). Similar results were obtained in midlactation cows treated with recombinant bST, where the response was an increase in responsiveness (+527.1%), with no effect in the sensitivity to the response (Sechen *et al.*, 1990). The increase in NEFA response to epinephrine correlated ($r = 0.82$) with the elevated circulating somatotropin concentrations induced by differing protocols for administration of 25 IU pituitary-derived bST in late lactation (McCutcheon & Bauman, 1986). In pigs the NEFA response to epinephrine was not enhanced in a similar fashion to cows, as a decrease in the very high rate of lipogenesis was most likely the target for adaptation rather than lipolysis (Wray-Cahen *et al.*, 1993). The glycerol (lipolytic) response to epinephrine was enhanced in cows treated with 40 mg/d recombinant bST, which was an increase in the responsiveness of glycerol to epinephrine (+182.0%) without any change in sensitivity of the response (Sechen *et al.*, 1990).

The NEFA response to **insulin challenge** was also greatly altered by exogenous bST. Where insulin challenge failed to affect NEFA in control cows (+5.5 Mcal/d energy balance) NEFA concentration was decreased by insulin challenge in treatment cows (–5.4

Mcal/d energy balance) with similar results for the glycerol response to insulin challenge (Sechen *et al.*, 1990). Similar results were observed during **glucose challenge**, where no distinct response was reported following challenge in control cows, with a clear reduction in bST treated cows (Sechen *et al.*, 1989). There was no effect of pituitary-derived bST treatment of early lactation cows on the NEFA responses to **glucagon challenge** (Sechen *et al.*, 1989).

Following daily injections of midlactation dairy cows with pituitary-derived bST, a 74.1% increase in the irreversible loss rate of NEFA occurred, where the absolute amount of NEFA oxidized was almost doubled (Bauman *et al.*, 1988). The proportion of total NEFA used for oxidation was increased by 24.7% during 2 weeks of pituitary-derived bST treatment, with a large increase in the proportion of CO_2 derived from NEFA oxidation (from 3.5% to 6.4% of total CO_2), an increase of 82.9% (Bauman *et al.*, 1988). On the second day of 0.1 IU/kg body weight pituitary-derived bST treatment the irreversible loss rate of NEFA (as represented by palmitate) was unaffected at peak lactation, but increased by 20.2% at midlactation where the NEFA concentration was also doubled (McDowell *et al.*, 1987b). The arterio-venous difference for NEFA over the hindlimb was unaffected by pituitary-derived bST treatment, while the percentage NEFA extraction tended ($P < 0.10$) to decrease by 36.1% (McDowell *et al.*, 1987a). The arterio-venous difference for NEFA across the mammary gland was greatly enhanced (by +213.9% of control) by short-term pituitary-derived bST treatment, while the percentage extraction of NEFA tended to increase ($P < 0.10$) by 92.4% (McDowell *et al.*, 1987a).

2.3.4 Protein metabolism

The effects of exogenous bST on the metabolism of proteins or amino acids during lactation are far less well documented than the effects on lipids and carbohydrates. When high-producing dairy cows (103 days postpartum) were in a negative nitrogen balance, 14 days of treatment with pituitary-derived bST decreased nitrogen balance by 61.9% of control, although the statistical significance of this change was unclear (Tyrrell *et al.*, 1988). In multiparous Holstein cows long-term recombinant bST treatment (for 9 weeks, from 9 weeks postpartum) decreased protein balance by 108 g/d, which lead these cows to develop a negative protein balance, when control cows had achieved a positive protein

balance, whereas body protein gain remained unaffected (Chilliard *et al.*, 1991). Administration of recombinant bST to cows increased calculated protein gain between week 20 and week 39 postpartum (5.8 kg more than the close to zero values of control cows), when fortnightly treatment with recombinant bST was initiated at 9 weeks postpartum and pasture feeding occurred from week 24 postpartum (Chilliard *et al.*, 1991). In this experiment, protein gain over the entire recombinant bST treatment period (week 9 to week 39 postpartum) was 5.0 kg/d greater than control cows (Chilliard *et al.*, 1991). A shortage of amino acid supply can explain the lack of response in milk protein yield and reduction in milk protein content (while milk nitrogen was increased) in these cows (Tyrrell *et al.*, 1988).

The circulating **urea concentration** was not affected by 0.1 IU/kg body weight pituitary-derived bST treatment around peak, but decreased by 36.4% by treatment in midlactation, where no effect on urea irreversible loss rate occurred (McDowell *et al.*, 1987b). A similar reduction of 29.8% was observed in mid to late lactation cows on a nutrient rich diet, where excess net energy and crude protein were available (McGuire *et al.*, 1992). In growing steers a 24.5% reduction in plasma urea concentration occurred in response to 13 days of recombinant bST treatment (Boisclair *et al.*, 1994). An acute effect of recombinant bST on equine blood urea-nitrogen was observed as a reduction of approximately 40% by day 2 after injection of a slow-release 500 mg preparation, which had gradually returned to baseline by 16 days after injection (Buonomo *et al.*, 1996). There was no effect of slow-release recombinant bST exposure in midlactation cows on the expression of hepatic enzymes of the urea cycle, *carbamoyl phosphate synthetase*, *argininosuccinate synthetase*, or *ornithine transcarbamylase* (Velez & Donkin, 2004).

The efficiency of transfer of amino acids from arterial blood to the mammary gland was unaffected by exogenous bST treatment between week 5 to 20 postpartum, with no effect on the concentrations of individual amino acids or the arterio-venous differences (Austin *et al.*, 1991). In growing steers the 40.2% increase in retained nitrogen was due to a 20.2% decrease in nitrogen loss in urine (Boisclair *et al.*, 1994).

2.4 Effect on enzymes

Early lactation is characterized by decreased insulin biological effect and increased β-adrenergic responses, with marked effects on lipogenesis and lipolysis of adipose tissue, sustained over the lactational period by adaptations in the sympathetic nervous system (reviewed by McNamara, 1995). Somatotropin administration has been shown to have insulin antagonistic effects (e.g. decreased adiposity), insulin agonistic effects (e.g. body weight gain) and effects independent of insulin (e.g. circulating lipoproteins) in rats (Frick *et al.*, 2002). Differences in tissue priority for lipid synthesis make extrapolation of data from humans and rats to ruminant animals and pigs difficult. In rats somatotropin has a liver-specific lipogenic effect, whereas insulin has an adipose-specific lipogenic effect (Frick *et al.*, 2002). In somatotropin deficient dwarf rats exposure to physiological recombinant human somatotropin that restored growth and IGF-I failed to affect the basal and insulin-stimulated glucose transport, glucose transporter (GLUT) 1 and GLUT 4 content or the activity of enzymes like *citrate synthase*, *lactate dehydrogenase* and *β-3-hydroxyacyl-coenzyme A (CoA) dehydrogenase* in skeletal muscle (Daugaard *et al.*, 1999).

Decreased lipogenesis in pigs (anti-insulin effects) induced by somatotropin were not associated with altered insulin binding, receptor affinity or insulin receptor *tyrosine kinase* activity (Magri *et al.*, 1990). Postreceptor anti-insulin effects of somatotropin on adipocytes could be mediated at the level of *phosphatidylinositol phospholipase C* induction by insulin, through inhibition of guanosine triphosphate binding protein (G protein) activation of the enzyme through Gs, but not G_i content as described in *ob/ob* mice (Roupas *et al.*, 1991).

The major effects of exogenous somatotropin administration are localized in adipose tissue, specifically involving the enzymes of lipogenesis and lipolysis. There were less consistent or no effects on the activity of metabolic enzymes in muscle tissue (Daugaard *et al.*, 1999), the liver (Rizza *et al.*, 1981, Adriaens *et al.*, 1992) or the mammary gland (Lanna *et al.*, 1995, Liesman *et al.*, 1995, Beswick & Kennelly, 1998, Beswick & Kennelly, 2000).

2.4.1 Gluconeogenesis

The production of milk necessitates an obligatory rise in hepatic glucose output through gluconeogenesis, which generally functions at a high rate due to increased substrate supply. It was estimated that an additional 0.38 mol glucose was required by the gland for every kg milk produced (Danfær, 1994). Processes associated with mobilization of body reserves establish conditions favourable to gluconeogenesis like increased hepatic mitochondrial amino acids and acetyl-CoA (favours pyruvate conversion to oxaloacetate), with decreased glucose and a low insulin to glucagon ratio, while increased glucose flux could also be associated with increased somatotropin (Danfær, 1994). Although it has been suggested that exogenous somatotropin administration did not alter the gluconeogenic capacity of cows, it should be considered that this was estimated from the hepatic mRNA content for *phosphoenolpyruvate carboxykinase* and *pyruvate carboxylase* where treatment period was only 7 days (Pershing *et al.*, 2002). There was also no effect of 5 weeks of recombinant bST treatment on hepatic *pyruvate kinase* expression (Velez & Donkin, 2004). Similarly no effect of 2 months of recombinant bST treatment of midlactation cows was reported on gluconeogenesis per gram tissue (Liesman *et al.*, 1995), but treatment has also been shown to be associated with a 14.0% increase in liver weight (Binelli *et al.*, 1995). The capacity for hepatic gluconeogenesis (and oxidation) from propionate was increased by 62.9% in response to 11 days of pituitary-derived bST treatment in midlactation cows (Pocius & Herbein, 1986) and by 94.5% ($P<0.06$) in cows treated for 8 weeks with recombinant bST (Knapp *et al.*, 1992). This effect could be facilitated by the 32.1% increase in *phosphoenolpyruvate carboxykinase* expression in liver samples from midlactation cows treated with slow-release recombinant bST for 5 weeks, which was not significant at 3 weeks after initiation of treatment (Velez & Donkin, 2004). The endogenous hepatic output of glucose determined by tracer studies in hyperinsulinaemic euglycaemic clamp was unaffected by treatment of either early or late lactation Holstein cows with growth hormone-releasing hormone (Rose *et al.*, 1996).

2.4.2 Lipogenesis

Increased milk yield in the absence of compositional changes necessitates greater fatty acid availability that could arise from the diet, increased *de novo* synthesis by the gland or increased supply of preformed (mobilized) long-chain fatty acids (Beswick & Kennelly,

1998). As intake is not generally acutely affected and no evidence was found of increased *de novo* fatty acid synthesis in primiparous cows that displayed an increase in milk fat yield, additional fatty acids had to arise from mobilized NEFA (Beswick & Kennelly, 1998).

In the **mammary gland** the activity of enzymes of lipid metabolism like *lipoprotein lipase* (Azzara & Dimick, 1987, Liesman *et al.*, 1995, Beswick & Kennelly, 1998), *acetyl-CoA carboxylase*, *fatty acid synthase* (Beswick & Kennelly, 1998), and *stearoyl-CoA desaturase* (Beswick & Kennelly, 2000) remained unaffected by administration of exogenous somatotropin. The activity of *lipoprotein lipase* (in milk and mammary tissue), the utilization of acetate for *de novo* fatty acid synthesis (Lough *et al.*, 1989, Liesman *et al.*, 1995) and acetate oxidation by mammary tissue extracts from cows treated with exogenous somatotropin were also unaffected by treatment (Lough *et al.*, 1989). The effects of recombinant bST in midlactation cows were ascribed to partitioning effects on lipids rather than altered mammary metabolism, mediated by decreased *de novo* fatty acid synthesis (Beswick & Kennelly, 1998) and fatty acid uptake from lipoproteins in adipose tissue (Beswick & Kennelly, 2000). There is however an increase in RNA content and accretion of mammary parenchyma, which suggests a greater metabolic activity and milk synthesis per cell (Binelli *et al.*, 1995).

Adipose tissue fatty acid synthesis from acetate was greatly decreased (–96.6%) by 2 months of recombinant bST exposure in midlactation cows (Liesman *et al.*, 1995). Similarly the overall rate of lipogenesis was decreased by 96.9% in cows that were initially in a significantly positive energy balance (Lanna *et al.*, 1995). In midlactation cows exposed to recombinant bST for 63 days adipose tissue *acetyl-CoA carboxylase* and *fatty acid synthase* mRNA abundance decreased to undetectable levels (Beswick & Kennelly, 1998), with undetectable *stearoyl-CoA desaturase* mRNA concentration and a decrease of 85.4% in tissue *lipoprotein lipase* mRNA (Beswick & Kennelly, 2000) and a 72.3% decrease in *lipoprotein lipase* activity (Liesman *et al.*, 1995). Similarly exposure to recombinant bST for only 8 days decreased total *acetyl-CoA carboxylase* (–88.1%) and *fatty acid synthase* (–68.9%) activity (Lanna *et al.*, 1995). There was a milder decrease in the pathways that generate reduced nicotinamide adenine dinucleotide phosphate (NADPH) and *glucose-6-phosphate*

dehydrogenase activity was decreased by 49.6%, while the reductions in *6-phosphogluconate dehydrogenase* and *isocitrate dehydrogenase* activity did not reach statistical significance (Lanna *et al.*, 1995).

Lactation in ewes was characterized by decreased activity of lipogenic enzymes like *acetyl-CoA carboxylase*, *fatty acid synthase*, *glucose 6-phosphate dehydrogenase* and *6-phosphogluconate dehydrogenase* in adipose tissue, while the addition of insulin (and dexamethasone) to culture media increased the activity of these enzymes (Vernon *et al.*, 1991). Somatotropin prevented the *in vitro* insulin-induced induction of *acetyl-CoA carboxylase* and *glucose 6-phosphate dehydrogenase*, but did not significantly affect insulin-induced *fatty acid synthase* or *6-phosphogluconate dehydrogenase* activity in adipose tissue of nonlactating and lactating sheep (Vernon *et al.*, 1991).

In growing pigs a large proportion of glucose is generally channelled to adipose tissue for lipogenesis and the reduction in lipid accretion induced by somatotropin treatment has been ascribed principally to decreased lipogenesis (Walton *et al.*, 1987, Magri *et al.*, 1990), due in a large part to decreased *acetyl-CoA carboxylase* activity (Liu *et al.*, 1994). Exposure of barrows to pituitary-derived pST for 7 days decreased the basal uptake and responsiveness of insulin-stimulated uptake of glucose by 62.0% and 47% respectively, which was closely paralleled by the 63.3% reduction in basal lipogenesis as estimated by carbon labelled [^{14}C] glucose incorporation into fatty acids (Magri *et al.*, 1990). Similarly basal adipose tissue lipogenesis, the responsiveness and sensitivity of lipogenesis to insulin and IGF-I were greatly decreased by 1 week of pituitary-derived pST administration in growing barrows (Walton *et al.*, 1987). *Acetyl-CoA carboxylase* mRNA abundance was decreased by 42.1% in adipose tissue of growing pigs treated with daily intramuscular injections of recombinant pST for 51 days (Liu *et al.*, 1994). Like in lactating cows the *fatty acid synthase* activity of adipose tissue was decreased to non-detectable levels by 7 days of pituitary-derived pST treatment of barrows (Magri *et al.*, 1990). NADPH production in the pentose-phosphate pathway and pyruvate-malate cycle was also attenuated through decreased *glucose 6-phosphate dehydrogenase* (–48.9%), *6-phosphogluconate dehydrogenase* (–10.7%) and *malate dehydrogenase* (–62.0%) activity (Magri *et al.*, 1990).

2.4.3 Lipolysis and oxidation

Lipid oxidation (β-oxidation and ketogenesis) depends principally on the availability of fatty acids in mitochondria and the rate of transport across mitochondrial membranes (Danfær, 1994). These processes are therefore favoured by mobilization of body reserves and the accumulation of the product of β-oxidation (acetyl-CoA) will also favour increased gluconeogenesis. Although somatotropin is generally not thought to greatly enhance lipolysis, a possible increase in G_S signal transduction would enhance the response of *hormone-sensitive lipase* to lipolytic signals (Roupas *et al.*, 1991). The activity of *hormone-sensitive lipase* was increased by as much as 55.2% compared to the almost complete inhibition of lipogenesis in adipose tissue of dairy cows exposed to recombinant bST (Liesman *et al.*, 1995). The effects of recombinant bST on lipolytic rate in cows seemed primarily to be the result of an indirect response, through altered responses to inhibitory effectors of the β-adrenergic signal transduction pathway that include decreased responsiveness and/or sensitivity to inhibition by adenosine (Lanna *et al.*, 1995). Maximal β-agonist stimulated lipolytic rate was increased along with the number of β-adrenergic receptors, while the ability of an adenosine analogue and prostaglandin E_2 to inhibit this response was greatly decreased with no effect on their receptors or G protein numbers (Doris *et al.*, 1996).

2.5 General responses

2.5.1 Feed intake and digestibility

Treatment of dairy cows with exogenous bST at various stages of lactation for less than 5 weeks consistently failed to stimulate an increase in feed intake (Peel *et al.*, 1981, Bauman *et al.*, 1985a). A 5% decrease in feed intake was observed in dairy cows at 192 days postpartum treated with a very high dose of pituitary-derived bST (100 IU/d), where these cows were experiencing a significant drop in energy balance and a rise in plasma NEFA concentration (Eppard *et al.*, 1985b). Six weeks of exposure to slow-release recombinant bST increased feed intake by 5.0% (Velez & Donkin, 2004), while long-term treatment of pasture-fed cows increased intake by 7.7% at 8 weeks of treatment, which continued to increase and reached +13.6% by 22 weeks of 39 IU/d pituitary-derived bST treatment (Peel *et al.*, 1985). At 9 to 11 weeks of 37.8 IU/d and 56.7 IU/d recombinant

bST treatment the dry matter intake was 15.0 % higher than control, while the effects from 12 to 16 weeks of treatment were confounded by dietary adjustments (Bauman *et al.*, 1985a). The difference in intake was sustained over several lactations and in the fourth lactation of recombinant bST treatment the treatment cows had a 10.9% greater intake than control at approximately 120 days postpartum, when cows were treated from 60 days postpartum (Adriaens *et al.*, 1992).

Although an increase in digestibility due to pituitary-derived bST administration has been suggested, the effect was small and only significant in one week (Peel *et al.*, 1985), while the purity of the pituitary extracts can be questioned especially considering the risk of contamination with prolactin, which was not tested. No effect on the apparent digestibility of feed was observed after 2 weeks of pituitary-derived bST exposure, although a 9.3% increase in methane production occurred, which contributed to the small decrease in ME intake of these cows (Tyrrell *et al.*, 1988). Similarly no effect on the intake or digestibility of either dry matter or nitrogen was observed in growing steers treated with recombinant bST for 16 days (Boisclair *et al.*, 1994). Surprisingly exposure to daily subcutaneous injections of bST between weeks 5 to week 20 postpartum decreased the total yield of volatile fatty acids with altered molar proportions of individual volatile fatty acids, including a reduction in propionate and an increase in acetate (Austin *et al.*, 1991). However the sampling protocol for collection of ruminal fluid was brought in question and could have yielded erroneous results (Austin *et al.*, 1991).

2.5.2 Body weight and energy balance

Acute exposure to exogenous bST failed to affect the body weight of midlactation (McDowell *et al.*, 1987b) or early lactation (McDowell *et al.*, 1987a, Rose *et al.*, 2005) cows, while significant changes in body weight were generally very small (McDowell *et al.*, 1987b, Tyrrell *et al.*, 1988). Milk production and metabolic effects of recombinant bST administration were maintained in primiparous cows where the additional adaptations of growth were present, with a 17.2% increase in milk yield, no change in body weight gain, increased carcass protein percentage (+6.6%) and decreased carcass lipid (–44.5%) in response to recombinant bST (Binelli *et al.*, 1995). Only a small decrease in body weight (2.1% of control) occurred when cows were treated with 51.5 IU/d pituitary-derived bST

(McDowell *et al.*, 1987b) and even with long-term exposure to exogenous bST body weight (Austin *et al.*, 1991) and body weight gain remained unaffected (Bauman *et al.*, 1985a, Peel *et al.*, 1985). However a tendency ($P < 0.10$) toward greater body weight gain was observed in cows treated with recombinant bST for 30 weeks, with a tendency ($P < 0.10$) toward decreased BCS gain of 0.52 units (Chilliard *et al.*, 1991). Over this extended period of treatment cows experienced a significant increase in body water and protein, and the loss of lipid (–22.8 kg) tended to reach significance ($P < 0.10$) compared to a small gain in control cows (Chilliard *et al.*, 1991). This altered composition of body weight gain could be considered unfavourable, although it may also contribute to preventing over-conditioning of cows.

Exposure to exogenous bST decreased the energy balance of lactating dairy cows when milk production increased acutely in the absence of a feed intake response and even presented when cows in a positive energy balance were chronically treated and intake increased (Bauman *et al.*, 1985a). Even when cows were in a significantly negative energy balance (–11.0 Mcal digestible energy/d) at 74 days postpartum, a further reduction in energy balance (to –23.9 Mcal/d) occurred with exposure to pituitary-derived bST for 10 days (Peel *et al.*, 1981). Oxygen (O_2) consumption was increased by 4.1% by 14 days of pituitary-derived bST treatment and tissue energy mobilization was greatly enhanced, being the origin of the increased milk energy output in treated cows (Tyrrell *et al.*, 1988).

Fortnightly recombinant bST administration (500 mg slow-release) from 9 weeks postpartum failed to affect the loss of body lipid between week 7 and week 20 postpartum, or the gain of lipid between weeks 20 to week 39 postpartum (Chilliard *et al.*, 1991). However, over the entire treatment recombinant bST period (difference between week 9 and week 39), recombinant bST affected a overall loss of body lipid compared to a gain in control cows, with a tendency ($P < 0.10$) to decrease lipid accretion by 22.8 kg over 30 weeks of lactation (Chilliard *et al.*, 1991).

2.5.3 Insulin-like growth factors and other hormones

Chronic treatment (22 weeks) of cows with 39 IU pituitary-derived bST per day increased the serum concentration of IGFs by 214.0%, while the increase in IGF failed to reach statistical significance at week 8 of treatment (Peel *et al.*, 1985). The plasma IGF-I concentration increased by as much as 200% (Cisse *et al.*, 1991, Molento *et al.*, 2002) and 300% (Rose *et al.*, 2005) with the administration of recombinant bST in early lactation Holstein cows and this response was enhanced by a further 88.8% above somatotropin alone, when insulin treatment also occurred (Molento *et al.*, 2002). A 153.3% increase in hepatic IGF-I mRNA was not accompanied by any change in the number of hepatic somatotropin receptors in recombinant bST treated midlactation cows (Velez & Donkin, 2004). The size of the milk production response correlated positively with the IGF-I response ($r = 0.546$) in early lactation cows treated with recombinant bST for 2 weeks (Rose *et al.*, 2005). Administration of recombinant bovine or pST to horses increased the IGF-I concentration several-fold, reaching a peak by the fifth and last day of injection (Buonomo *et al.*, 1996). There was however no effect of treatment with recombinant pST in nursing piglets, probably due to a lack of maturity of the somatotropic axis (Dunshea *et al.*, 1999).

Poor nutrition is characterized by elevated somatotropin concentrations and somatotropin resistance, with a lack of IGF-I response (reviewed by Gluckman *et al.*, 1987) and in early lactation dairy cows IGF-I concentrations correlated ($r = 0.59$) with energy balance (Cisse *et al.*, 1991). In well-fed midlactation cows a single recombinant bST challenge resulted in a 100% increase in plasma IGF-I concentration at 18 to 24 hours after injection, which failed to develop when cows were deprived of feed (McGuire *et al.*, 1995a). A 3 day treatment of late lactation (195 days postpartum) Holstein cows tended to increase basal IGF-I concentration by 25.4%, while the increase did not reach statistical significance in early lactation (Rose *et al.*, 1996). In dairy cows where the effects of recombinant bST and nutrition were investigated, exogenous somatotropin elevated circulating IGF-I concentrations, where the highest concentrations were observed in cows fed the most nutrient dense diet (Newbold *et al.*, 1997), or in cows consuming a diet formulated to 120% of calculated requirement compared to limiting either crude protein and/or net energy to 80% of requirements (McGuire *et al.*, 1992). The response of IGF-I to

recombinant bST also tended ($P<0.06$) to be lower in early lactation dairy cows with a somewhat negative energy balance, than the responses in late lactation and the dry period when nutrient balances were positive (Vicini *et al.*, 1991).

The binding proteins for IGF play an important role in modulating the biological efficacy of circulating IGF-I, with facilitating and inhibitory functions among the different types of IGFBP (reviewed by (Breier, 1999). IGFBP 3 is the most abundant and is decreased by low nutrition, while IGFBP 2 was increased by a reduction in nutritional status (Breier, 1999). In piglets before weaning, where the somatotropic axis had not reached maturity yet there was no effect of recombinant pST treatment on IGFBP 3 although a reduction in response to low feeding plane was noted (Dunshea *et al.*, 1999). The IGFBP 2 concentration was increased in early lactation compared to late lactation or dry period cows, which could be related to the slightly negative calculated energy and crude protein balance during this period (Vicini *et al.*, 1991). Treatment of late pregnant or lactating cows with recombinant bST resulted in a decrease in the IGFBP 2 concentration and an apparent increase in IGFBP 3 (Vicini *et al.*, 1991). Exogenous bST and pST administration in mares also resulted in a general increase in IGFBPs corresponding to IGFBP 1, IGFBP 3 and IGFBP 4 (Buonomo *et al.*, 1996). Nutrient restriction to 80% of the nutrient requirements (for net energy and crude protein) also decreased the IGFBP 2 response to recombinant bST with a smaller reduction when nutrient intake was restricted (McGuire *et al.*, 1995a).

A 10-day treatment with pituitary-derived bST increased serum prolactin concentration by 51.5% (Peel *et al.*, 1981), most likely an effect of contamination of the pituitary extract. The use of highly-purified bST extracts or recombinant bST did not affect the concentrations of thyroid hormones (Peel *et al.*, 1982, Bitman *et al.*, 1984, Cisse *et al.*, 1991), prolactin (Peel *et al.*, 1982, Pocius & Herbein, 1986), or cortisol in dairy cows (Peel *et al.*, 1982).

2.5.4 General health

Treatment of dairy cows with exogenous bST did not affect the somatic cell count (Bauman *et al.*, 1985a), rectal temperature (Tyrrell *et al.*, 1988, Adriaens *et al.*, 1992) or reproductive health of dairy cows (Bauman *et al.*, 1985a, Eppard *et al.*, 1985b), without an apparent effect on the health of the calves born to cows (Eppard *et al.*, 1985b). The ability to sustain enhanced milk yield (up to +41.2%) over as much as 100 days of lactation (Bauman *et al.*, 1985a) and by +24.4% after 4 consecutive lactations of recombinant bST (Adriaens *et al.*, 1992) suggests that exogenous somatotropin administration did not lead to stress. In dairy ewes recombinant bST injections over the entire lactational period caused an increase in somatic cell count (+74.3%) from midlactation onward, although this was not associated with an increase in the prevalence of bacteriologically positive samples, or a greater incidence of subclinical mastitis (Brozos *et al.*, 1998). Exposure to 100 IU/d pituitary-derived bST for 10 days increased efficiency of milk production at the expense of mobilized body tissue, with a greater body weight loss and reduction in energy balance than the usual application of 50 IU/d (Eppard *et al.*, 1985b). Coupled to a reduction in intake and a potentially very large cumulative loss of body lipid and protein (Eppard *et al.*, 1985b), this high-dose application of exogenous somatotropin could potentially have harmful effects over extended periods of treatment.

2.5.5 Heart function and blood flow

Although long-term exposure to recombinant bST over 4 lactations did not significantly affect heart rate or respiration rate (Adriaens *et al.*, 1992), heart rate of cows treated with pituitary-derived bST for 2 weeks tended to be 7.7% greater ($P < 0.10$) than control cows at approximately 103 days postpartum (Tyrrell *et al.*, 1988). The weights of organs like the cardiac ventricles, lungs, intestines and kidneys were increased by between 5 to 15% by recombinant bST administration to primiparous Holstein cows (Binelli *et al.*, 1995).

Blood flow to the hindlimb (skeletal muscle) was unaffected by 0.14 IU/kg body weight, subcutaneous pituitary-derived bST injection for 3 or 4 days in lactating cows (McDowell *et al.*, 1987a) or intramuscular recombinant bST in growing steers (Boisclair *et al.*, 1994). However estimated mammary blood flow was increased by 28.7% by 0.14 IU/kg body weight, pituitary-derived bST (McDowell *et al.*, 1987a). The mammary blood flow

correlated strongly (r = 0.897) with milk yield, while the blood flow to milk yield ratio (520:1) was unaffected by short-term treatment with pituitary-derived bST (McDowell *et al.*, 1987a). The increase in blood flow to the mammary gland could be coincidental with the increased metabolic activity of the gland, but could also be due to local vasodilatory responses mediated by the gland itself (Breier *et al.*, 1991). It was however suggested that the milk production response was not the result of increased mammary blood flow, but rather that the enhanced metabolic status of the gland induced the increase in blood flow (Bell & Bauman, 1997). Decreased involution and/or cell proliferation does not seem to occur, as parenchymal weight and DNA content remained unaffected by recombinant bST administration, while increased metabolic activity was suggested by the increase in RNA content of cells (Binelli *et al.*, 1995). This increased metabolic activity of the gland could then also explain some of the metabolic changes observed in the absence of any apparent effect on enzyme activity (Beswick & Kennelly, 1998).

3. Other responses in lactation

Optimizing productive efficiency of dairy cows requires identification of the factors that determine efficiency and the means (genetic and environmental) by which they can be improved (Bauman *et al.*, 1985b). Although cows of a good BCS that maintain body weight postpartum ran less metabolic risks postpartum, a loss of body weight with the associated risk of ketosis nearing peak resulted in greater milk production in the on-farm environment (Busato *et al.*, 2002). A thorough understanding of the physiological adaptations of lactation and the factors that affect these are necessary to formulate optimal nutritional, management and reproductive strategies for high-production dairy cows, especially during the transition period (Bell, 1995).

3.1 Production and general responses

3.1.1 Nutrient supplementation

There was no difference in the dry matter intake of high-production dairy cows on high quality diets supplemented with either protein (Komaragiri *et al.*, 1997) or fat (Komaragiri *et al.*, 1998), which also failed to induce milk yield responses. The postruminal supply of glucose and sodium-caseinate, failed to significantly increase milk production, or enhance the pituitary-derived bST induced increase in milk production of well-fed dairy cows, even though the energy supply was increased by 25.9% (Peel *et al.*, 1982). However supplementation of the diet with casein and branched-chain amino acids in combination with 4 days of insulin infusion increased milk production by 12.4% and milk protein yield by 25% (with an 11% increase in milk protein content), suggesting that the potential for milk protein synthesis is not fully expressed in dairy cows (Mackle *et al.*, 1999). This increase in milk protein occurred at the expense of milk fat and milk lactose, both of which were decreased in these late lactation cows (Mackle *et al.*, 1999). In cows fed corn silage *ad libitum* and a calculated amount of concentrate the high concentrate group failed to show improved milk production, while body weight and body protein loss were accentuated (15.7 kg and 2.9 kg greater respectively) by the fact that the crude protein intake was actually decreased by supplementation (Chilliard *et al.*, 1991). A reduction in either net energy or crude protein to 80% of the calculated requirements in lactating cows decreased milk production in a similar manner, while a reduction in both components lead to a further reduction in production, which was characterized by increased milk fat percentage and decreased milk protein percentage (McGuire *et al.*, 1992). A greater than 20% reduction in net energy intake decreased milk production by 18.4% during the first 8 weeks postpartum when cows were milked twice daily, but only by 11.9% when cows were milked three times daily (Andersen *et al.*, 2004). The ability to synthesis lactose was not enhanced by postruminal glucose supply in Holstein cows fed a high quality total mixed ration, while the slight increase in protein yield (+6.4%) in response to protein supply did not reach statistical significance (Peel *et al.*, 1982).

There are physiological limitations to milk production in high-producing dairy cows and the realization of the production potential of the gland depends primarily on the delivery of nutrients to the mammary gland (reviewed by Bines & Hart, 1982). On high quality

rations the already-high milk production was not affected by additional glucose supply to the mammary gland (Peel *et al.*, 1982), lipid feeding (Komaragiri *et al.*, 1998, Blum *et al.*, 1999), protein supplementation of the diet (Komaragiri *et al.*, 1997), or postruminal protein supply (Peel *et al.*, 1982). The limitations to production did not seem to be alimentary in these studies, but rather related to the ability to mobilize large amounts of body reserves (Komaragiri *et al.*, 1997, Komaragiri *et al.*, 1998, Blum *et al.*, 1999) or the ability to synthesize lactose (supply of glucose precursors). The alimentary supply of glucose and fatty acids presents the principal metabolic challenge to nutrient availability to the gland and they are supplied though metabolic adaptations and body store mobilization (Bines & Hart, 1982). There is however still considerable room for improvement in the productive efficiency of dairy cows, especially through a greater understanding of nutrient partitioning and delivery to particular tissues and how these could be manipulated or optimized (reviewed by Bauman *et al.*, 1985b). The full development of the milk production response depends heavily on the supply of good quality nutrition, as the amount of hepatic binding sites for somatotropin (i.e. the normal hepatic and IGF-I response) is increased by increased nutrient density in feed (Newbold *et al.*, 1997). There was however no effect of a reduction in net energy and/or crude protein intake to 80% of requirements on the milk IGF-I content (McGuire *et al.*, 1992).

3.1.2 Genetic selection

Very little variation between cows exists in maintenance requirements, the ability to digest and absorb nutrients, or the ability to utilize ME for milk production (reviewed by Bauman *et al.*, 1985b). In Holstein heifers and cows selected on the basis of milk fat plus protein yield for 15 years, dry matter intake (of high-concentrate and low-concentrate feed) was similar to animals selected to the national average, whereas selection cows were still able to produce 12.0% (cows) and 20.8% (heifers) more milk (Veerkamp *et al.*, 1994). There was a mild interaction between genotype and concentrate feeding level for milk yield, suggesting that the genetically superior cow can suffer pronounced reductions in milk yield when nutritional management is not optimal (Veerkamp *et al.*, 1994). However, very little genotype-environment interaction currently exists, with only trends toward differences between superior *vs.* average cows (Veerkamp *et al.*, 1994). Selection increased the gross efficiency for milk energy (milk energy ÷ energy intake) and efficiency for milk

protein yield (milk protein yield ÷ crude protein intake), as the yield of milk increased, without changes in dry matter intake (Veerkamp *et al.*, 1994). In selected cows, the efficiency of milk production (kg milk yield ÷ kg dry matter intake) was improved by 16.2%, from 0.68 to 0.79 in first lactation Holstein cows (Reinecke *et al.*, 1993). These differences in efficiency are in part due to the dilution of maintenance costs and the increased contribution of body reserves to milk production (Bauman *et al.*, 1985b, Veerkamp *et al.*, 1994, Veerkamp & Emmans, 1995) although other advantages in selected cows exist (Veerkamp & Emmans, 1995). Milk composition for milk fat percentage, or milk protein percentage was not affected by selection for yield (kg) of fat plus protein over 15 years, although the yield of protein plus fat increased by 11.9% with a concurrent 12.0% increase in yield (Veerkamp *et al.*, 1994). In first lactation Holstein cows the increase of milk yield associated with selection occurred at the expense of a significant ($P < 0.01$) decrease in milk fat percentage (Reinecke *et al.*, 1993).

Although there does not seem to be a digestive or metabolic advantage in cows with a high genetic potential for milk production, their ability to partition nutrients toward productive processes seemed to be more efficient than their lower producing counterparts (Bauman *et al.*, 1985b, Veerkamp & Emmans, 1995). In first lactation cows genetically superior for milk production, dry matter intake was increased by an average of 12.3% from 45 days to 315 days postpartum, with a significantly greater milk yield over the lactational period (Reinecke *et al.*, 1993). Selection over 25 years lead to a 3 800 kg increase in milk yield over 305 days of lactation and was coupled to a 45.1% greater milk yield at 100 days postpartum in first lactation Holstein cows (Burmeister *et al.*, 1993).

Selection for milk solids over 15 years did not result in changes in the average body weight of heifers or cows over 26 weeks of lactation, but did lead to a 4.8% to 5.9% reduction in their average BCS (Veerkamp *et al.*, 1994). This indicates a greater loss of BCS in genetically superior animals, especially after peak lactation and is more pronounced for superior cows fed low-concentrate diets (Veerkamp *et al.*, 1994). In genetically superior first lactation Holstein cows a 3.5% greater body weight was observed compared to control cows, while a significant interaction of time postpartum was not elaborated on further (Reinecke *et al.*, 1993).

3.1.3 Body tissue mobilization

Care should be taken when interpreting data where cows of varying adiposity are used, especially considering that in one experiment control cows, although with a similar BCS to treatment cows, had a 33.0 kg greater empty body fat or 20.7% difference in adiposity (Komaragiri *et al.*, 1998). This difference in body fat content will clearly have profound effects on the nutrient mobilization and circulating nutrients of these "control" cows and bias all data collected. In studies by Komaragiri *et al.*, one unit change in body condition score (BCS) was analogue to 42 kg (Komaragiri *et al.*, 1997) and 54.8 kg (Komaragiri *et al.*, 1998) empty body fat in Holstein cows. In dairy cows of varying stages of lactation and production levels one unit BCS was compared to 35 kg body fat (Waltner *et al.*, 1994) or 29.2 kg body lipid and 44 kg body weight, corrected for gut fill (Chilliard *et al.*, 1991). Although 20 to 25% lower estimated net energy intake failed to affect body weight loss in the first 8 weeks of lactation when cows were milked twice daily, the loss of body weight (but not condition) was reduced by 15.7% in cows milked three times daily receiving the high net energy diet (Andersen *et al.*, 2004). Increased strain was placed on body reserves when cows were milked three times daily, where the body weight and BCS loss in the first 8 weeks postpartum was more than 20% greater than cows milked twice daily and remained above 10% even when net energy intake was greatly enhanced (Andersen *et al.*, 2004).

Very early in lactation, high-producing dairy cows mobilize both lipid and protein reserves in an attempt to correct the negative energy balance and nitrogen balance induced by the high irreversible loss of energy and protein in milk. The primary source of body weight loss in early lactation dairy cows is the body fat reserves (93% to 99%), while body protein makes a smaller (1% to 7%) contribution (Komaragiri *et al.*, 1997, Komaragiri *et al.*, 1998). For every kg body weight loss from 2 weeks prepartum through early lactation, 0.28 kg constituted water, 0.64 kg fat and only 0.08 kg protein, as determined by deuterium oxide analyses of body composition (Komaragiri *et al.*, 1997). From 2 weeks before parturition to 5 weeks postpartum high-producing Holstein cows mobilized 21 kg body protein, which was related to a 22.2% (Komaragiri *et al.*, 1997) and 15.8% (Komaragiri *et al.*, 1998) decrease, while between 47 kg body fat, or –33.1% (Komaragiri *et al.*, 1998) and 54 kg body fat, or –32.2% (Komaragiri *et al.*, 1997) was mobilized in this

7 week period. From 5 weeks to 12 weeks postpartum no further protein mobilization occurred in high-producing dairy cows, while lipid mobilization was slowed to a loss of only another 18 kg fat, or to –43.3% of 2 weeks prepartum (Komaragiri *et al.*, 1997). In a similar experiment no further changes in empty body lipid or protein were observed between 5 weeks and 12 weeks postpartum, but was accompanied by an increase in empty body weight by 18 kg over the 7-week period (Komaragiri *et al.*, 1998). The difference in calculated body lipid content between week 1 and week 7 postpartum in Holstein cows was 34.7 kg, while absolute body weight loss was only 18.7 kg, masked by the gain of 15.4 kg water (Chilliard *et al.*, 1991).

3.2 Metabolic responses

3.2.1 Plasma hormone and metabolite concentrations

Care should be taken when interpreting differences in the absolute concentration of hormones between cows (Bauman *et al.*, 1985b), as a concentration is not necessarily directly related to biological efficacy and even nutrient concentration does not estimate availability of that nutrient. Glucose is the most important nutrient of lactation and output from gluconeogenesis generally increases 4-fold from maintenance, but can potentially be as high as 7-fold in very high-producing dairy cows (Bell & Bauman, 1997). Glucose homeostasis and homeorhetic adaptations of glucose supply and demand are therefore of critical importance during lactation (Bauman & Currie, 1980). This is especially true during early lactation and becomes less important later in lactation, when the need for glucose in oxidation and lactose synthesis decreases as milk production declines. An estimated extra 0.38 mol/d glucose output from the liver required for every kg milk produced (Danfær, 1994). Important factors to consider with regard to nutrient partitioning are the stage of lactation, nutritional quality and genetic potential of cows. Glucose supply is maintained by a decrease in glucose utilization and increased substrate output by adipose tissue, but more importantly skeletal muscle amino acid mobilization (Bell & Bauman, 1997). The major adaptations of lactation include reduced responses of glucose utilization, lipogenesis and lipolysis to insulin and increased lipolytic responses to β-adrenergic stimulation (Bell, 1995).

The baseline plasma **glucose** concentration prior to bovine hyperinsulinaemic euglycaemic clamp was 6.1% higher in week 19 of lactation, compared to week 9 (Blum *et al.*, 1999) and 4.8% higher at 194 days postpartum compared to 35 days postpartum (Rose *et al.*, 1996). However, in beef cows producing only an average of 11.2 kg milk per day, the glucose concentration was similar in very early lactation (week 2 to week 5 postpartum), late pregnancy (week 8 to week 3 prepartum) and the dry period at 50.7 mg/dL whole-blood (Sano *et al.*, 1991). There was no difference between the whole-blood glucose concentrations of midlactation (144 days postpartum) Holstein cows *vs.* dry cows at 50 mg/dL *vs.* 53 mg/dL respectively (Sano *et al.*, 1993). In ewes fed *ad libitum*, the glucose concentration of early lactation was 11.5% lower than the dry period and was increased by late lactation to a value of –5.6% of the dry period, while ewes were gaining body weight (Metcalf & Weekes, 1990). A difference in glucose concentration of lactation failed to present when ewes were fed a restricted diet that lead to body weight loss in the lactation period (Metcalf & Weekes, 1990). However, limiting net energy intake that failed to effect body weight or body condition loss in early lactation lead to a small (6.6 to 7.6%) but significant decrease in blood glucose concentration (Andersen *et al.*, 2004). When cows were milked three times daily the blood glucose concentration decreased by 5.8 to 6.8% compared to cows milked twice daily, where blood glucose concentration was between 59.1 to 64.0 mg/dL (Andersen *et al.*, 2004).

A reduction in glucose concentration of a lower net energy intake was coupled to an overall increase of 18.8 to 26.2% in the **β-hydroxybutyrate** concentration in early lactation dairy cows, while increased milking frequency (where glycaemia was decreased) increased the β-hydroxybutyrate concentration by between 15.9 to 23.1% (Andersen *et al.*, 2004). Neither net energy intake nor milking three times daily affected the circulating NEFA concentration, most likely because the metabolic responses and change in energy balance to treatments were small (Andersen *et al.*, 2004).

Although **insulin** itself did not seem to have a direct regulatory role in mammary production of lactose or lipid, it affected the synthesis of milk protein through either direct effects or indirectly through the somatotropic axis and IGF-I in particular (McGuire *et al.*, 1995b). There was no difference in the insulin concentration at week 9 *vs.* week 19

(Blum *et al.*, 1999) and day 35 *vs.* 194 (Rose *et al.*, 1996) of lactation in dairy cows. In beef cows, the plasma insulin concentration (33.0 μU/mL) of very early lactation, late pregnancy and the dry period was similar (Sano *et al.*, 1991). Selection was associated with decreased insulin concentration of lactation in first lactation Holstein cows (Reinecke *et al.*, 1993) although the reduced insulin concentration could be the result of a relative nutrient shortage (akin to underfeeding) in higher yielding cows (reviewed by Bauman *et al.*, 1985b). In dry, nonpregnant or early pregnant Holstein cows the insulin concentration of plasma was similar to cows at 144 days postpartum at approximately 24 μU/mL (Sano *et al.*, 1993). There was no difference in the insulin concentration in plasma between lactational periods and the dry period in lactating ewes, with no effect of restricted intake on the insulin concentrations (Metcalf & Weekes, 1990). Although insulin concentration seemed to increase over the lactation, the differences did not reach statistical significance (Metcalf & Weekes, 1990). Relative hypoglycaemia was established by a reduction in net energy intake in the face of approximately 50% lower plasma insulin concentration (Andersen *et al.*, 2004).

There was a greater **somatotropin** concentration in plasma of genetically superior Holstein cows in their first lactation, compared to control cows (Reinecke *et al.*, 1993). However, these increased somatotropin concentrations were coupled to 18.2% lower mean IGF-I concentrations in cows of a greater genetic merit for milk production (Reinecke *et al.*, 1993), which suggests that differences could have been due to a metabolic bias of the results due to relative underfeeding and/or greater body reserves (Bauman *et al.*, 1985b). Decreased energy intake increased the overall somatotropin concentration (+111.1%), while IGF-I concentration was decreased by 36.8%, with no differences between cows three times daily or twice daily (Andersen *et al.*, 2004).

3.2.2 Results of the insulin challenge

Selection for 25 years in Holstein cows (+45.1% milk yield at 100 days) failed to elicit any reaction in the maximum response of glucose or the sensitivity of the half-maximal glucose response to insulin challenges of varying doses, from 0.125 to 4 μg/kg body weight (Burmeister *et al.*, 1993). Although the dose-response curves were generated by insulin challenges and not the usual insulin clamps, it is interesting to note the failure of response

in glucose homeostasis even in the face of a large change in genetic merit for milk production.

3.2.3 Results of the hyperglycaemic clamp

There was no difference between either the insulin or glucose metabolic responses of the hyperglycaemic clamp between week 9 and week 19 of lactation, in high-producing dairy cows (Blum *et al.*, 1999). However, in beef cows the plateau insulin concentration was 39.0% greater in early lactation, indicating a greater pancreatic response to 50 mg/dL whole-blood hyperglycaemia, when milk production was only 11.2 kg/d (Sano *et al.*, 1991). In these beef cows, the greater glucose turnover of lactation (+44.4% GIR) was still associated with a large insulin response, as less strain was placed on glucose homeostatic mechanisms in beef cows (Sano *et al.*, 1991). In dairy cows the MPII of lactation was 343.5% lower than in dry cows and the GIR to attain hyperglycaemia tended to be ($P < 0.10$) 37.0% lower in lactation, although this decrease in pancreas response was confounded by poor application of hyperglycaemic clamp protocols (Sano *et al.*, 1993). The MPII over GIR (MPII ÷ GIR) tended to decrease ($P < 0.10$, –200%) during lactation in Holstein cows (Sano *et al.*, 1993). However, hyperglycaemia was only reached at 50 minutes after glucose infusion started (t50), where insulin concentration was only significantly increased between t30 to t60 and not during the "steady-state" period, which included the whole of the last hour of 120 minute infusion where insulin concentration was not stable (Sano *et al.*, 1993). Similar problems with the application of metabolic tests in ruminant animals were evident in (Sano *et al.*, 1991). In high-producing dairy cows, feeding of triacylglycerol (220 g/kg dry matter) or NEFA (200 g/kg dry matter) did not affect hyperglycaemic clamp SSGIR, baseline plasma glucose concentration, baseline plasma insulin concentration, or plateau insulin concentration (Blum *et al.*, 1999).

3.2.4 Results of the hyperinsulinaemic euglycaemic clamp

The hyperinsulinaemic euglycaemic clamp SSGIR or sensitivity of whole-body glucose utilization of dairy cows was unaffected at week 19 of lactation compared to week 9, where similar baseline insulin concentrations were observed (Blum *et al.*, 1999). Similarly there were no significant differences between cows at peak and during late lactation for

estimates of glucose turnover (Rose *et al.*, 1996). However, the plateau insulin concentration was markedly increased (+33.7%), indicative of a greater pancreas response to exogenous glucose infusion at week 19, at 6 IU/kg×min insulin (Blum *et al.*, 1999).

In beef cows, the GIR in the last hour of the hyperinsulinaemic euglycaemic clamp (6.0 mU/kg×min) in very early lactation and late pregnancy was similar to the rate in the dry, nonpregnant period (Sano *et al.*, 1991). However, the sensitivity of peripheral tissues was increased by 35.3% in lactation compared to pregnancy, whereas plateau insulin concentrations were similarly decreased compared to the dry period (Sano *et al.*, 1991). During hyperinsulinaemic euglycaemic clamp (6.0 mU/kg×min) in Holstein cows the MPII was 42.8% lower during lactation compared to dry cows, although the lack of a priming dose meant that insulin concentration still seemed to be increasing in lactating cows at t120 (Sano *et al.*, 1993). The insulin metabolic clearance rate was significantly increased in lactating cows compared to dry nonpregnant or early pregnant cows, although this data was not reported and in the hyperinsulinaemic clamp euglycaemia was not effectively maintained (Sano *et al.*, 1993). In ewes, lactation failed to elicit a response in the basal glucose metabolic clearance rate, maximum glucose metabolic clearance rate or basal endogenous glucose appearance (Metcalf & Weekes, 1990). However, both glucose metabolic clearance rate and glucose endogenous appearance were more sensitive to insulin in lactation than the dry period (Metcalf & Weekes, 1990). Chronic physiological hyperinsulinaemia (5.2-fold baseline concentration) where glycaemia and milk production was maintained, failed to alter milk lactose and milk fat in the face of a 29% reduction in feed intake, while a small increase (+7.1%) in milk protein yield occurred (Bergman *et al.*, 1985).

The feeding of triacylglycerol or NEFA as a greater part of the energy requirements of high-producing dairy cows, did not affect the GIR or MPII of the hyperinsulinaemic euglycaemic clamp at week 9 or 19 of lactation (Blum *et al.*, 1999).

4. Mechanism of somatotropin response

4.1 Introduction

The effects of somatotropin treatment during lactation have been extensively reviewed and have lead to the conclusion that somatotropin is the principle homeorhetic adaptation that orchestrates and coordinates glucose metabolism in lactation (Bell & Bauman, 1997). The physiological adaptations of genetically superior cows that allow them to produce more milk are similar to the coordinated changes of metabolism in cows treated with bST (Peel & Bauman, 1987). This allows the producer to keep production levels ahead of the normal genetic potential of the herd (with proper management) and the producer with genetically superior animals to achieve even greater gains in yield. As a homeorhetic hormone (Bauman & Currie, 1980), somatotropin affects a whole range of physiological processes in several different tissues, with important nutrient partitioning effects (metabolic effects) and effects on cell number (somatogenic effects) and size (Etherton & Bauman, 1998).

The dose of pituitary-derived bST most effective in dairy cows is approximately 50 IU/d, as 5 IU and 10 IU did not affect production responses (except a small change in efficiency of milk production), while 25 IU lead to intermediate responses (Eppard *et al.*, 1985b). Application of 50 IU increased responses significantly, while 100 IU only resulted in a 7.8% advantage in the efficiency of milk production, where a negative energy balance and increased NEFA concentration was observed in dairy cows at 192 days postpartum (Eppard *et al.*, 1985b). The effects on production did not last beyond the period of administration (Peel *et al.*, 1982) and declined with the fall in endogenous somatotropin. Long-term administration of pituitary-derived bST did not sustain production to the same extent as recombinant bST (Bauman *et al.*, 1985a).

Efficacy of treatment with somatotropin was limited by the already-elevated endogenous somatotropin concentrations very early in lactation (Andersen *et al.*, 2004) and the smaller percentage increase in somatotropin concentration established by treatment (McDowell *et al.*, 1987b), where metabolic adaptations of later lactation were notably absent (Rose *et al.*, 1996). Although the same authors suggested that the very negative energy balance limited the response to somatotropin (McDowell *et al.*, 1987b), the

response was still evident in cows that were in a very negative energy balance after peak (Peel *et al.*, 1981). The size of the milk yield response to short-term recombinant bST administration was not affected by the parity of cows in multiparous Holstein cows (Rose *et al.*, 2005), while the effects of bST and improved nutritional quality were additive (Austin *et al.*, 1991).

4.2 Nutrients

Treatment with somatotropin alters the physiological responses to homeostatic signals for carbohydrate, lipid and protein metabolism, making "excess nutrients" available for production. Somatotropin caused a shift in the partitioning of nutrients toward milk production, even in the high yielding, early lactation dairy cow where large partitioning effects were already placing strain on metabolism (Peel *et al.*, 1981, Sechen *et al.*, 1989). A nutrient sparing effect can be observed as an increase in the nutrient concentration in the general circulation. In the lactating animal, this increase in metabolite concentration was often absent, because of the mammary gland's ability to extract nutrients from the bloodstream. These adaptations did not include changes in the digestive ability of the animal or nutrient absorption, but rather altered utilization of the absorbed nutrients as repartitioning away from storage and toward milk production (at the expense of body reserves) occurred (Tyrrell *et al.*, 1988). Major responses were related to decreased utilization of glucose and NEFA by the hindlimb of cows (i.e. skeletal muscle), with increased utilization of NEFA by the lactating mammary gland (McDowell *et al.*, 1987a), whereas responses in growing pigs were mainly due to decreased lipid accretion by adipose tissue due to decreased basal and insulin-stimulated lipogenesis (Walton *et al.*, 1987). Somatotropin coordinates both the supply of nutrients to the mammary gland and the utilization of these nutrients (partitioning) to allow a dramatic increase in the synthesis of milk components (Peel & Bauman, 1987, Etherton & Bauman, 1998). The "anti-insulin" effects of somatotropin include decreased glucose uptake and (insulin-stimulated) metabolism in adipose tissue and skeletal muscle, coupled to increased hepatic gluconeogenesis, which is relatively resistant to insulin inhibition (Bell & Bauman, 1997). The supply of extra nutrients did not mimic the effect of bST treatment on milk production, nor did the combination of pituitary-derived bST and nutrient supply enhance the bST response (Peel *et al.*, 1982).

Somatotropin does not increase the absolute efficiency of the animal, for example pituitary-derived bST did not affect the efficiency of energy use for maintenance, or the efficiency of the use of nutrients for milk synthesis (Tyrrell *et al.*, 1988). However, in short-term studies the gross efficiency of milk production was increased, probably only due to a reduction in the proportion of energy used for maintenance compared to production and the ability of the mammary gland to efficiently utilize the excess nutrients mobilized from peripheral tissues (Bauman *et al.*, 1985a, Tyrrell *et al.*, 1988). However, once intake was increased by exogenous somatotropin, the gross efficiency of milk production returned to control (Bauman *et al.*, 1985a, Peel *et al.*, 1985), but it is still possible that somatotropin can have subtle indirect effects on the digestive ability and/or maintenance requirements of animals (Breier *et al.*, 1991).

The galactopoietic effect of bST clearly reaches beyond nutrient partitioning, however this did not include a direct effect on the mammary gland, as locally infused pituitary-derived bST (up to 1920 IU/d) had no effect on milk yield or composition in sheep and goats (McDowell *et al.*, 1987c). A small, non-significant increase in milk production occurred at 3840 IU pituitary-derived bST per day, most likely due to the increase in somatotropin concentration in plasma as the contralateral control half exhibited a similar increase in production (McDowell *et al.*, 1987c). The effects of somatotropin treatment on dairy animals are mediated by the pronounced increase in IGF-I observed (Peel *et al.*, 1985) as well as direct effects of somatotropin on peripheral tissues. At 86 days postpartum the administration of insulin with recombinant bST enhanced the increase in IGF-I concentration of plasma (Molento *et al.*, 2002).

4.3 Metabolic changes

4.3.1 Carbohydrate metabolism

The responses most extensively researched are associated with the changes in carbohydrate (and more specifically glucose) metabolism, because an increase in milk production necessitates a parallel increase in glucose supply for lactose synthesis in the mammary gland. The insulin-induced utilization of glucose by tissues was reduced by exogenous bST administration (Sechen *et al.*, 1989, Sechen *et al.*, 1990, Molento *et al.*,

2002), while pancreatic response to glucose remained unaffected (Sechen *et al.*, 1989, Sechen *et al.*, 1990). The glucose response to epinephrine challenge remained unaffected by pituitary-derived bST treatment (Sechen *et al.*, 1989). The reduction of glucose utilization and particularly oxidation (Bauman *et al.*, 1988) was associated with a reduction in utilization of glucose by muscle (McDowell *et al.*, 1987a), as very little glucose was utilized by ruminant adipocytes. Glucose partitioning away from muscle tissue toward the lactating mammary gland was the result of postreceptor changes, as some insulin effects were enhanced and others decreased (Sechen *et al.*, 1990). It seemed that the major effects of somatotropin occurred at the levels of maximal responsiveness to insulin instead of the basal response or sensitivity of the glucose responses to insulin in dairy cows (Rose *et al.*, 1996) and growing wethers (Rose & Obara, 1996). The gluconeogenic enzyme concentration (estimated by hepatic mRNA concentration) was not affected by 7 days of bST exposure in early lactation (Pershing *et al.*, 2002).

4.3.2 Lipid metabolism

The effects of somatotropin administration on lipid metabolism were most pronounced of the major nutrients. Exogenous somatotropin enhanced the lipolytic effects of the catecholamines, but apparently not the glycogenolytic or gluconeogenic effects (McCutcheon & Bauman, 1986, Sechen *et al.*, 1989). Increased lipolytic responses to epinephrine were most likely due to postreceptor changes in the epinephrine signal transduction pathway, as illustrated by the maximum response of glycerol to varying doses of epinephrine challenge (Sechen *et al.*, 1990). An additional reduction in the re-esterification of fatty acids was observed, as a far greater increase in NEFA responsiveness to epinephrine was observed, i.e. the increase in the response of glycerol, or lipolysis alone was less than the increase in the response of NEFA, or the sum of an increase in lipolysis and decrease in lipogenesis (Sechen *et al.*, 1990). The NEFA and glycerol response to insulin challenge was greatly enhanced and recombinant bST enhanced the antilipolytic effect of insulin, when treatment cows were in a negative energy balance (Sechen *et al.*, 1990). Effect in growing pigs were noticeably different, especially for lipid metabolism and was thought to reflect differences in the physiological state leading to homeorhetic adaptations and the contrary state of lipid turnover in pigs (Wray-Cahen *et al.*, 1993).

Milk energy increased at the expense of tissue energy (Tyrrell *et al.*, 1988). The amount (mol/d) NEFA oxidized almost doubled (Bauman *et al.*, 1988), i.e. a large proportion of body energy was derived from β-oxidation. To facilitate changes in carbohydrate distribution body tissues utilize lipid as the preferred substrate rather than glucose. Lipid oxidation was increased by 92.5%, which was accompanied a 19.4% reduction in glucose oxidation (Bauman *et al.*, 1988), with a greater utilization of mobilized fatty acids by the mammary gland (McDowell *et al.*, 1987a). The increase in NEFA response AUC following epinephrine challenge correlated with the milk energy excretion ($r = 0.82$) and the fat yield ($r = 0.95$), even though the NEFA concentration and positive energy balance remained unaffected (McCutcheon & Bauman, 1986).

The effects of exogenous somatotropin on lipid metabolism depend on the nutrient balance of animals. Although negative energy and nitrogen balance do not prevent lactational responses, they lead to altered milk composition upon treatment with bST (Peel *et al.*, 1981, Bitman *et al.*, 1984, Eppard *et al.*, 1985b, Tyrrell *et al.*, 1988). When treatments were initiated in cows in a negative energy balance the milk fat percentage was consistently decreased (Peel *et al.*, 1981, Richard *et al.*, 1985, Tyrrell *et al.*, 1988) and also in cows where a positive energy balance was greatly decreased (Eppard *et al.*, 1985b) or to negative levels (Eppard *et al.*, 1985b, Sechen *et al.*, 1989, Sechen *et al.*, 1990). When bST administration had a lipolytic effect (increased circulating NEFA concentration), the absolute amount of NEFA removed by mammary tissue was greatly enhanced (McDowell *et al.*, 1987a) and preformed fatty acids incorporated into milk lipid at an accelerated rate (Bitman *et al.*, 1984, Eppard *et al.*, 1985a).

4.3.3 Protein metabolism

The ability to sustain milk protein secretion depends on the protein balance and a negative nitrogen balance (Tyrrell *et al.*, 1988) and even a reduction in a positive balance by as little as 4 g/d can lead to a reduction in milk protein content when milk production increased (Eppard *et al.*, 1985a, Eppard *et al.*, 1985b). Due to the complex nature of protein metabolism, few studies have examined the effects of somatotropin and measurements of circulating concentrations are confounded by the presence of constituents in milk. Milk nitrogen was increased although milk protein content decreased

(Tyrrell *et al.*, 1988), while a reduction in milk protein content was associated with a reduction in milk urea-nitrogen (Molento *et al.*, 2002).

4.3.4 Cardiovascular changes

Another important adaptation of lactation is the increase in mammary blood flow brought about by the increase in cardiac output. Additionally, a larger proportion of the cardiac output is directed toward the mammary gland, most likely regulated automatically by the increase in metabolism (Bell & Bauman, 1997) and/or other adaptive responses of the mammary gland (Breier *et al.*, 1991). In addition to partitioning effects, the increase in blood flow toward the mammary gland itself will directly affect production, as these parameters were highly correlated (McDowell *et al.*, 1987a).

4.4 Summary

Treatment of dairy cows with exogenous somatotropin resulted in increased milk production by as much as 40% in cows where the increases in milk yield, milk lipid content and milk protein content were accommodated exclusively by increased availability of nutrients from tissue mobilization and nutrient repartitioning (Sechen *et al.*, 1990). Treatment for at least 5 weeks was required to initiate significant intake responses (Velez & Donkin, 2004) with no apparent effect on digestibility (Tyrrell *et al.*, 1988, Boisclair *et al.*, 1994). An increase in the gross efficiency of cows of 10% to 30% generally occurred during acute treatment periods with pituitary-derived bST (Eppard *et al.*, 1985b, Tyrrell *et al.*, 1988, Sechen *et al.*, 1990) or recombinant bST (Peel *et al.*, 1981, Peel *et al.*, 1982, Richard *et al.*, 1985). The responses of body weight mobilization and increased intake seemed to be coordinated, as cows generally failed to exhibit a significant change in body weight or body weight gain even in primiparous cows (Binelli *et al.*, 1995), although the composition of body weight gain favoured protein over lipid accretion (Chilliard *et al.*, 1991, Binelli *et al.*, 1995). Prolonged exposure of cows to exogenous bST did not seem to affect the general or reproductive health of cows (Bauman *et al.*, 1985a) or their calves (Eppard *et al.*, 1985b).

The very large demand for nutrients for milk production was generally met without changes in circulating glucose or insulin concentration, but rather through altered glucose homeostatic responses in insulin-sensitive tissues in the face of unaltered insulin responses (Sechen *et al.*, 1989, Sechen *et al.*, 1990). There were decreased responses of glucose to insulin challenge (Sechen *et al.*, 1990) and hyperinsulinaemic euglycaemic clamp (Molento *et al.*, 2002) especially measures of glucose clearance where treatment later in lactation was more effective (Rose *et al.*, 1996), most likely due to the extensive homeorhetic mechanisms that were already in place in early lactation cows. There was however little effect in dairy cows treated with bST on glucose responses to glucose challenge (Sechen *et al.*, 1989, Adriaens *et al.*, 1992), epinephrine challenge (McCutcheon & Bauman, 1986, Sechen *et al.*, 1989) or glucagon challenge (Sechen *et al.*, 1989) suggesting that insulin biological efficacy is the principle homeorhetic adaptation of glucose metabolism brought about by somatotropin. Basal glucose irreversible loss rate was increased and, while the proportion of this glucose directed to lactose synthesis remained unaffected (McDowell *et al.*, 1987b, Bauman *et al.*, 1988), a smaller proportion of glucose was directed toward oxidation (Bauman *et al.*, 1988). The level of oxidation estimated from carbon dioxide (CO_2) production in tissues was maintained during bST treatment (Tyrrell *et al.*, 1988) by decreasing the contribution of glucose to total oxidation and by supplying alternative fuels (Bauman *et al.*, 1988) like NEFA to tissues (Bauman *et al.*, 1988, Sechen *et al.*, 1990). A major component of the glucose-sparing effects of bST treatment was a reduction in the utilization of glucose by the hindlimb (McDowell *et al.*, 1987a) and glucose availability was further enhanced by an increase in the release of glucose precursors that may include lactate (McDowell *et al.*, 1987a, Boisclair *et al.*, 1994), amino acids (Danfær, 1994) and glycerol (Sechen *et al.*, 1990). The capacity for hepatic gluconeogenesis was enhanced (Pocius & Herbein, 1986, Knapp *et al.*, 1992) and there was increased *phosphoenolpyruvate carboxykinase* expression following chronic exposure of lactating cows to bST (Velez & Donkin, 2004).

In addition to the increase in lipolysis in adipose tissue, there was also a reduction in lipogenesis that was particularly pronounced when a considerable decrease in energy balance of cows occurred (Sechen *et al.*, 1990). In contrast to glucose, the modulation of lipid metabolic responses were far more extensive with altered responses to insulin

(Sechen *et al.*, 1990) and glucose challenge (Sechen *et al.*, 1989) and enhanced responses to epinephrine (McCutcheon & Bauman, 1986, Sechen *et al.*, 1989, Sechen *et al.*, 1990), with no difference in glucagon challenge (Sechen *et al.*, 1989). The overall response in lipid metabolism to bST was to make a greater amount of NEFA available for oxidation in tissues (Bauman *et al.*, 1988) and more preformed fatty acids available for the formation of milk lipids (McDowell *et al.*, 1987a). There was a shift in the balance of metabolism in adipose tissue away from lipogenesis, favouring lipolysis. In adipose tissue uptake of lipoprotein fatty acids (Liesman *et al.*, 1995, Beswick & Kennelly, 2000), *de novo* fatty acid synthesis from acetate (Liesman *et al.*, 1995) and enzymes of lipogenesis like *acetyl-CoA carboxylase* and *fatty acid synthase* were decreased (Lanna *et al.*, 1995, Beswick & Kennelly, 2000). Fatty acid esterification remained unaffected (Liesman *et al.*, 1995), while the enzymes of NADPH production were decreased with a more clearly defined effect in the pentose-phosphate pathway than the *isocitrate dehydrogenase* pathway (Lanna *et al.*, 1995). A concurrent increase in lipolysis (Lanna *et al.*, 1995) through enhanced *hormone-sensitive lipase* (Lanna *et al.*, 1995, Liesman *et al.*, 1995) was established by bST treatment and was characterized by attenuated responses to inhibitors of β-adrenergic stimulation (Lanna *et al.*, 1995, Doris *et al.*, 1996) and an increase in the number of β-adrenergic receptors (Doris *et al.*, 1996).

Some of the metabolic responses to recombinant bST administration failed to fully develop when treatment was combined with nutrient restriction. For example the insulin-like growth factor-I (IGF-I) and IGF binding protein 2 (IGFBP 2) responses to bST treatment decreased in feed deprived cows (McGuire *et al.*, 1995a), while the percentage increase in milk production progressively decreased with a decrease in the nutrient density of the diet (Newbold *et al.*, 1997). Nutrient restriction had effects on glucose metabolism that were in direct opposition to homeorhetic adaptations, like increased complete and partial oxidation of glucose (Rhoades *et al.*, 2007) and decreased glucose production (Petterson *et al.*, 1993). However undernutrition also induced a reduction in metabolic clearance rate of glucose (Janes *et al.*, 1985, Petterson *et al.*, 1993) where the glucose-sparing response was induced to accommodate the reduction in alimentary precursors. Energy restriction in cows was characterized by somatotropin resistance, where the IGF-I response to somatotropin was uncoupled (Andersen *et al.*, 2004) and the amount of

hepatic somatotropin binding sites was decreased by a reduction in nutrient density of the diet (Newbold *et al.*, 1997).

Energy intake restriction or decreased energy density of the diet decreased circulating concentrations of insulin, glucose and IGF-I, but increased somatotropin, NEFA and β-hydroxybutyrate concentrations in lactating cows (Andersen *et al.*, 2004). Restriction to 50% of the predicted energy requirements failed to affect insulinaemia, but decreased the glucose concentration of pregnant, but not dry nonpregnant ewes (Petterson *et al.*, 1993). Supplementation of a dairy cow total mixed ration with postruminal glucose and casein did not affect milk yield or efficiency of milk production at 12 weeks postpartum, even though the additional nutrients increased the energy supply by 25.9% (Peel *et al.*, 1982) and lipid feeding did not significantly affect the metabolic responses of either glucose or insulin in lactating cows (Blum *et al.*, 1999). The supply of additional nutrients failed to affect the responses of cows to bST treatment (Peel *et al.*, 1982), while concentrate restriction that failed to significantly affect energy intake also did not affect the responses to bST treatment of lactating dairy cows (Cisse *et al.*, 1991). Glucose metabolic clearance rate was decreased by energy restriction to 50% of requirements in ewes, where basal hepatic glucose production and the maximal reduction in glucose production in response to insulin was decreased with no effect on the dose-response characteristics of SSGIR (Petterson *et al.*, 1993). The sensitivity of glucose metabolic clearance rate to hyperinsulinaemia was decreased in nutrient restricted lactating ewes (Metcalf & Weekes, 1990).

CHAPTER 3. MATERIALS AND METHODS

1. Animals

1.1 Introduction

Ten healthy, multiparous, Holstein cows from the University of Pretoria Experimental Farm dairy herd were used in the experiment (n=10) and formed part of the data analysis. Data collection encompassed a period from the first week postpartum (week 1), to the twelfth week postpartum (week 12) for each cow and is summarized in Figure 7. In the basal period from week 1 to week 7 infrequent blood sampling occurred fortnightly, on two consecutive days with a total of 8 plasma samples (paragraph 2.1). This was followed by an experimental period from week 8 to week 12, where treatments were applied and the responses to metabolic tests were determined. These metabolic tests (described below in 3.4 and 3.5) included an insulin challenge in the morning followed by a hyperinsulinaemic euglycaemic clamp later in the day, with a total of 21 plasma samples for each of the 4 experimental periods. During the experimental period the effects of recombinant bST administration and/or intake restriction on the results of metabolic tests were determined (paragraphs 2.2.1 through 2.2.4).

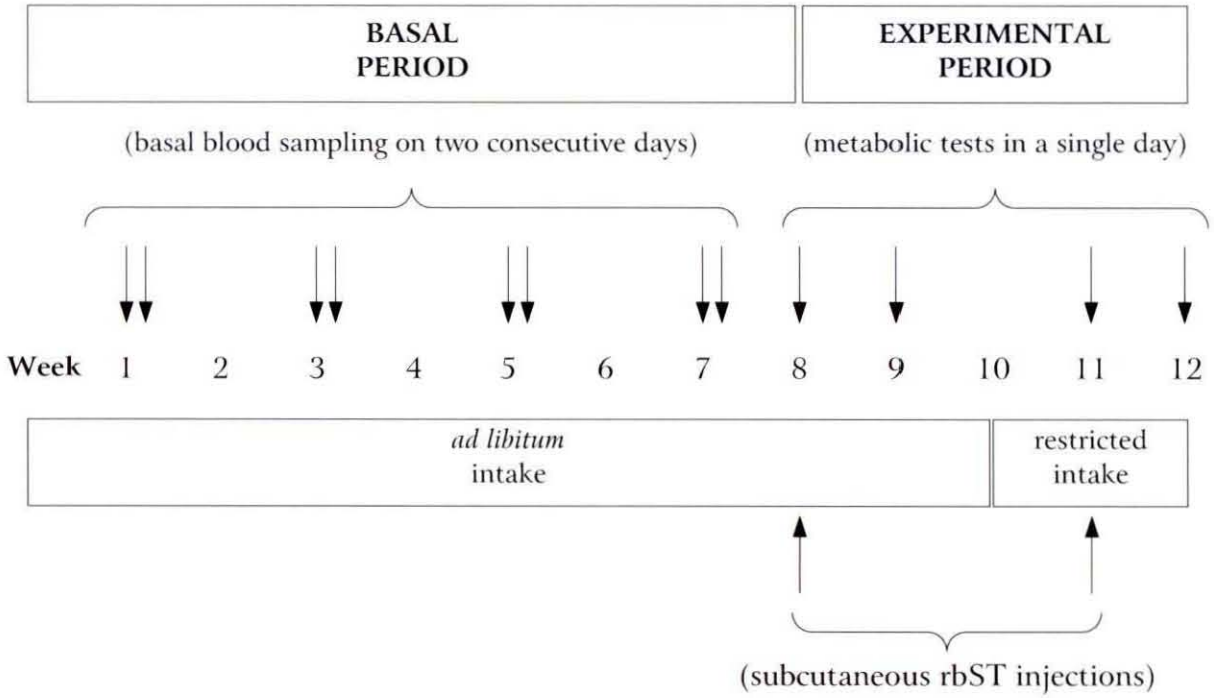

Figure 7. Diagrammatic representation of experimental protocol

1.2 Cows included in the data analysis

Cows were milked twice daily, the morning milking starting from approximately 5:00 and the afternoon milking from approximately 14:00. All cows were fed a commercial dairy ration, containing 7.6% whole cottonseed, 17.8% maize silage, 26.8% *Eragrostis curvula* hay and 47.8% high-protein concentrate (on a dry matter basis). The feed supplied an estimated 11.35 MJ ME per kg dry matter intake.

The BCS where 1 was emaciated and 5 obese, as well as age and parity of cows were used as criteria for inclusion in the experiment (Table 1). Determination of BCS, being a subjective measure of body fat, was determined in conjunction with technical personnel. All cows had a BCS between 3.0 to 3.5, were between 3.2 to 5.5 years of age and entering their second to fourth lactation. Cows in this herd were assigned three digit numbers, according to year and order of birth. For example, cow number 620 was born in 1996 and was the twentieth female calf born to the herd during that year. The data included in the data set was collected from May 2000 to April 2001.

Table 1. Description of cows included in the data set

No.	Cow	Calving Date	Sex of the Calf	Lactation	Age	BCS
1	**620**	22 May 2000	Female	Third	4.0	3.5
2	**515**	19 June 2000	Male	Fourth	5.2	3.1
3	**608**	19 June 2000	Male + Female	Third	4.3	3.0
4	**524**	30 June 2000	Male	Fourth	5.3	3.0
5	**606**	09 July 2000	Female	Third	4.5	3.3
6	**705**	21 August 2000	Female	Second	3.6	3.5
7	**631**	01 September 2000	Male	Third	4.1	3.2
8	**721**	25 November 2000	Female	Second	3.2	3.0
9	**543**	26 December 2000	Female	Fourth	5.1	3.3
10	**521**	13 January 2001	Male	Fourth	5.5	3.2

1.3 Cows excluded from the data analysis

Some cows were used to determine basal profiles of production and hormone secretion in the absence of metabolic tests and treatments, while testing the practical application of the procedures and setting up the equipment (Table 2). Cows that calved down from January 2000 to April 2000 included thirteen cows (No. 11 through 23) that were sampled according to the basal sampling procedure, up to the twelfth week postpartum. Fifteen cows were sampled, weighed and data recorded in the course of the entire experiment (from September 1999 to April 2001), but never used in further sample and data analyses. These cows were used during the planning phase of the experiment, fell ill early in experimental procedures or were included only as a safety margin, all of which lead to incomplete data collection.

Table 2. Description of cows excluded from the data set

No.	Cow	Calving Date	Sex of the Calf	Lactation	Age	BCS
11	543	17 January 2000	Female	Third	4.2	3.4
12	601	19 January 2000	Male	Third	4.0	3.5
13	405	25 January 2000	Male	Fifth	5.8	3.4
14	641	25 January 2000	Female	Second	3.1	3.3
15	535	03 February 2000	Female	Third	4.3	3.0
16	424	16 February 2000	Female	Fifth	5.6	3.4
17	421	19 February 2000	Female	Third	5.6	3.1
18	431	25 February 2000	Male	Fourth	5.5	3.4
19	439	04 March 2000	Male	Fourth	5.2	3.0
20	503	18 March 2000	Female	Fourth	5.1	3.0
21	427	28 March 2000	Male	Fourth	5.6	3.3
22	638	31 March 2000	Female	Second	3.4	3.0
23	536	01 April 2000	Male	Third	4.4	3.3
24	612	20 February 2000	Female	Third	3.7	3.1
25	527	19 June 2000	Female	Fourth	4.8	3.3
26	727	05 November 2000	Male	Second	3.0	3.0
27	643	26 November 2000	Female	Third	3.9	3.5
28	627	09 January 2001	Female (twins)	Third	4.5	3.4
29	715	26 November 2000	Female	Second	3.4	3.2
30	718	23 September 2000	Female	Second	3.2	3.4
31	710	20 October 2000	Female	Second	3.4	3.3

Eight cows sampled for more extensive periods were excluded for assorted reasons (No. 24 through 31 in Table 2). Cow number 612 was removed from the experiment in week 8 due to a foot abscess. Cow number 527 was only sampled up to 11 weeks postpartum due to scheduling difficulties. Cow number 727 exhibited a very poor response to insulin injection (Figure 8) and, because ketosis was suspected (Sakai *et al.*, 1996), was removed from the experiment. Cow 643 refused feed, after developing severe clinical mastitis in one quarter toward the end of data collection (week 12). This cow was subsequently removed from the data analysis. Due to poor temperament and nervousness in the crate, cow number 627 was excluded in week 9, when metabolic tests required frequent handling and intensive sample collection. Cow 715 was dropped after falling ill after 7 weeks of sampling and received veterinary treatment at Onderstepoort. During the initial insulin challenges of two cows (718 and 710) extremely low baseline glucose concentrations and severe insulin resistance was observed (Figure 8). It was suspected that these cows suffered from clinical ketosis and they were removed from the experiment with no further data collection.

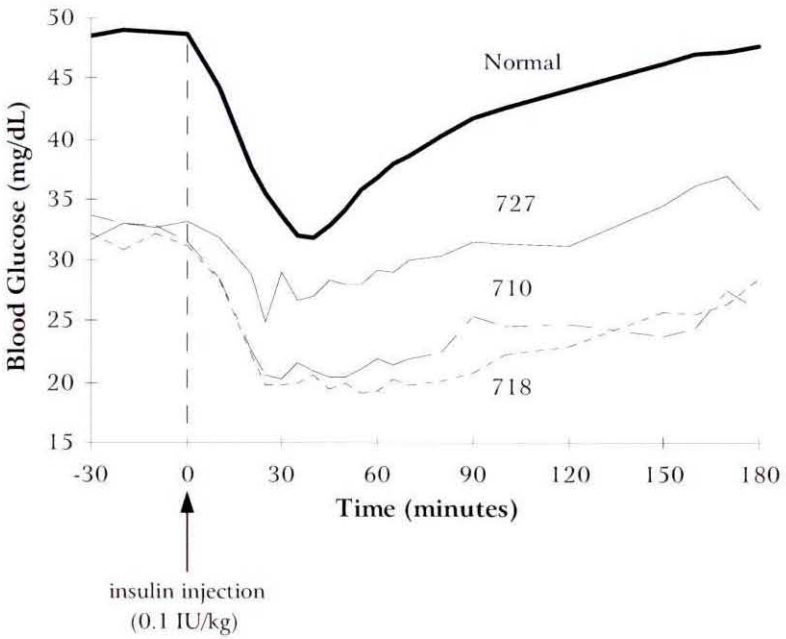

Figure 8. Insulin challenge results of cows with suspected ketosis

2. Periods and treatments

2.1 The basal period

In the basal period from week 1 to week 7 postpartum cows were exposed to standard management practices of the university. Although not subjected to any treatments, fortnightly blood samples were harvested on two consecutive days and frozen until analysis. The basal milk production was recorded with every milking and the body weight and BCS were recorded every week.

A total of 451 blood samples were collected for harvesting of plasma from all cows during the basal period. Following the basal period, insulin challenges and hyperglycaemic clamps with frequent blood sampling were initiated. A total of 64 metabolic tests were performed and 1 141 plasma samples harvested in 10 017 aliquots. Only 40 of these metabolic tests proved useful, with 4 test days for each of the 10 cows in the experiment.

2.2 The experimental period

2.2.1 The control period

The experimental period was initiated during week 8 of lactation, when samples were collected to serve as a control. Cows were subjected to insulin challenges followed by hyperglycaemic clamps during this week, while still subject to standard management practices of the herd.

2.2.2 The recombinant bovine somatotropin period (rbST)

Directly following collection of the control data (± day 56), cows received a subcutaneous injection of recombinant bST. During week 9 (± day 63, or 7 days after recombinant bST injection) metabolic tests were performed for collection of the data for the rbST period.

2.2.3 The restriction to 80% of metabolizable energy requirement period (80% ME)

During week 10 (± day 70), intake was restricted to 80% of the predicted ME requirements (NRC, 1988). From this time up to the end of the experiment, cows were isolated from the herd and individually fed a calculated amount of feed presented twice

daily. During week 11 (± day 77, or 7 days after energy restriction), metabolic tests were performed for the collection of the data for the 80% ME treatment period.

2.2.4 The 80% ME and rbST period (80% ME + rbST)

Collection of samples in the 80% ME period was immediately followed by subcutaneous injection of recombinant bST (± day 77). Cows were maintained on the restricted intake level and metabolic tests performed during week 12 (± day 84). This data was used to estimate the combined effects of the energy restriction and the recombinant bST treatments in the 80% ME + rbST period. The same experimental protocols were used in all cows and after the completion of these tests they were released back into the herd.

3. Data collection and processing

3.1 Body weight, body condition score and milk production

The nonfasting body weight and BCS of cows were recorded between 7:00 and 8:00, following the morning milkings. These observations were made on two consecutive days of every week (from week 1 to week 12 postpartum). Milk production was recorded for each cow at every milking, during the entire experiment. The weighted averages of the body weight, BCS and milk production were calculated for each week. Additionally, each cow was weighed after an overnight fast, prior to metabolic tests (weeks 8, 9, 11 and 12) to obtain the fasting body weight. These values were used to calculate the insulin injection volume and GIR.

3.2 Blood sampling

During the basal period venous blood samples were collected, occasionally following body weight and BCS measurements. The area of skin above the jugular vein was disinfected using ethanol and laboratory tissue paper. Blood was collected by jugular venipuncture into 10 mL heparinized, evacuated tubes (Becton Dickinson) that were gently inverted a few times and stored on ice-cold water until centrifugation (Hettich Zentifugen) at a gravitational force of 1 000 × g or approximately 3 200 rpm for 15 minutes. All blood

samples were centrifuged within one hour of collection, plasma transferred to seven marked 1.3 mL aliquots (DELT) and frozen at –20 °C until analysis.

During the experimental period, blood samples were collected with the aid of an indwelling catheter, using either 5 mL or 10 mL syringes (Promex). Whole-blood samples were collected into 1.3 mL aliquots containing approximately 1 mg ethylene diamine tetra-acetic acid (EDTA) disodium salt (Saarchem) per milliliter blood. These whole-blood samples were gently inverted to ensure proper mixing with anticoagulant and to prevent blood cells from settling out before analysis for glucose concentration within one minute of collection. Larger blood samples collected for harvesting of plasma were transferred to either 5 mL or 10 mL heparinized tubes (Becton Dickinson), gently inverted and placed on ice-cold water until centrifugation at 1 000 × *g* for 15 minutes. The levels of anticoagulants used were suitable for assays and the loss of glucose by glycolysis was minimized by storage on ice water, minimal delay before centrifugation (plasma) and rapid assay (whole-blood).

3.3 Catheterization

At 8, 9, 11 and 12 weeks postpartum, jugular catheters were inserted to facilitate frequent blood sampling during insulin challenges and hyperglycaemic clamps. A large area of skin around the jugular vein was washed using warm water, disinfectant and cloth. This area was dried with laboratory tissue paper and the area of insertion cleaned using ethanol and laboratory tissue paper. A hypodermic needle (14 × 1.5") was used to gain entry to the jugular vein and an indwelling catheter inserted at least 20 cm into the vein. The catheter material used was either polythene (Portex) or pure vinyl (Dural Plastics and Engineering). The contralateral jugular vein was also catheterized according to the same protocol, where one was used exclusively for blood sampling (usually on the right), while the other was used exclusively for infusion of solutions. Catheter integrity was maintained using a 25 IU/mL heparin solution (Novo Nordisk) in physiological saline and the catheters were plugged after insertion. Both catheters were firmly fixed to the skin, just above the point of entry, while the external loops were loosely secured close to the skin, to prevent damage. The cows were confined to a crush, before being moved to a crate inside a metabolic house for the remainder of the procedure.

3.4 Insulin challenge

After catheterization, cows were taken into a metabolic house and stalled in a raised crate with adjustable neck clamp until completion of the metabolic tests. The cows were frequently offered water, as the facility would not allow for unlimited access to water. Blood sampling commenced after at least one hour of confinement in the crate, when the whole-blood glucose concentration determined by frequent sampling stabilized. An insulin challenge (0.1 IU/kg body weight, 210 minutes) was conducted, with frequent determination of glucose in whole-blood and collection of plasma. More detail on sample collection during the insulin challenge is presented in Table 5. Insulin was injected as a commercial preparation of 40 IU/mL porcine insulin (Caninsulin®, Intervet) at a dose of 0.1 IU insulin per kg fasting body weight. Insulin was in suspension as Zn-insulin (30% amorphous and 70% crystalline) and was injected in a logarithmically decreasing manner, over a period of approximately one minute. Injection started immediately following collection of the final baseline blood sample, the time designated zero (t0). All insulin injections into the infusion catheter were followed by a saline flush, at least 4-fold greater than catheter volume.

3.5 Hyperglycaemic clamp

As soon as the whole-blood glucose concentration stabilized after the insulin challenge, a hyperglycaemic clamp (+50 mg/dL whole-blood, 120 minutes) was initiated, with frequent sampling of whole-blood and intermittent collection of plasma. More detail on sampling protocols during hyperglycaemic clamps is presented in Table 6. During the first 30 minutes following baseline observations, whole-blood glucose concentration was gradually raised to 50 mg/dL above the average baseline concentration, by injection of a 60% weight per volume (% $w/_V$) glucose solution. All glucose injections into infusion catheters were followed by a saline flush. Once the desired level of hyperglycaemia was achieved a 40% $w/_V$ glucose solution was infused at a variable rate to maintain the blood glucose concentration within a 10% range (± 10%) of this value.

The 60% w/v glucose solution (Saarchem) was prepared using glucose monohydrate ($G{\cdot}H_2O$) and distilled water (H_2O). Because the molecular weight of glucose is approximately 180 g/mol and water approximately 18 g/mol, it was calculated that only 91% of the $G{\cdot}H_2O$ was effectively glucose. The 60% w/v glucose solution was therefore made up by using 66 g $G{\cdot}H_2O$ filled to 100 mL with H_2O. For each clamp, 200 mL solution was prepared on the day preceding the clamp. During the first 30 minutes of clamped glucose (t0 to t30), the 60% w/v glucose solution was injected between blood collections using a 10 mL syringe. The volume of the injected solution was recorded every 5 minutes.

A 40% w/v glucose solution was prepared using 44 g $G{\cdot}H_2O$ filled to 100 mL using H_2O. For each clamp 500 mL solution was prepared on the day preceding the clamp and this was infused during the final 90 minutes of the clamp using a peristaltic pump (Ismatec). The weight of infused solution was recorded every 5 minutes and adjusted to maintain the whole-blood glucose concentration within the specified limits. These values were also used to calculate the GIR during the hyperglycaemic clamp.

After 2 hours of sustained hyperglycaemia, the catheters were removed, the area of skin around the insertion cleaned, recombinant bST injected when required (week 8 and week 11) and the cows were lead from the metabolic house to the milking parlour for the afternoon milking. After milking, animals on *ad libitum* intake were released back into the herd, while those on the restricted intake levels were separated from the herd for individual feeding.

3.6 Recombinant bovine somatotropin injection

After the metabolic tests performed in week 8 and 11, a small area of skin (postscapular) was cleaned using a commercial disinfectant and laboratory tissue. A subcutaneous recombinant bST injection was applied following the manufacturer's instructions. A commercial preparation of 1.4 mL recombinant bST in disposable syringes was used, containing Zn n-methionyl bST (Zn sometribove), equivalent to 500 mg bST (Lactotropin®, Elanco).

3.7 Calculations

3.7.1 Glucose infusion rate

The volume of the 60% w/v glucose solution was recorded every 5 minutes and was directly used in calculating the GIR. The weight of the 40% w/v solution, recorded every 5 minutes was used indirectly, to calculate the weight of glucose infused during this period. The weight of 100 mL of the 40% w/v glucose solution was measured as 114.7 g and 34.9 g glucose was effectively infused for every 100 g of this solution. The absolute rate of glucose infusion was calculated from the recorded values.

$$\text{Grams Glucose Infused} = 0.4 \times \frac{\text{Weight Solution Infused}}{1.147}$$

After calculation of the absolute rate of glucose infusion, the total weight of glucose infused was used to calculate the GIR as the weight glucose infused per minute, adjusted for the body weight of cows (i.e. mg / kg × min).

$$\text{Glucose Infusion Rate (GIR)} = \frac{\text{Total Weight Glucose Infused}}{\text{Fasting Body Weight} \times \text{Time}}$$

3.7.2 Glucose area under the curve

To evaluate the glucose response to insulin injection the area under the glucose concentration curve was calculated using trapezial geometry (Figure 9). Briefly, the AUC was calculated between each time point, or glucose sample collected (whole-blood and plasma). The formula used to calculate the area of a trapezium is:

$$\text{Area Under the Curve } (AUC_n) = \tfrac{1}{2} (y_0 + y_n) (x_n - x_0)$$

where y_0 = the glucose concentration in mg/dL at the first time point

y_n = the glucose concentration in mg/dL at the second time point

x_0 = the time in minutes of the first time point

x_n = the time in minutes of the second time point

The sum of the individual areas between time points was used to calculate the total AUC of the baseline preceding time zero (t–30 to t0) and the initial response after insulin injection (t0 to t30) for both whole-blood and plasma. The AUC value used for comparison of the response to insulin injection (t0 to t30) was a corrected AUC (Figure 9), calculated by correcting the response AUC for the baseline AUC, using the formula:

$$\text{Corrected AUC} = \text{AUC}_{t0 - t30} - \text{AUC}_{t-30 - t0}$$

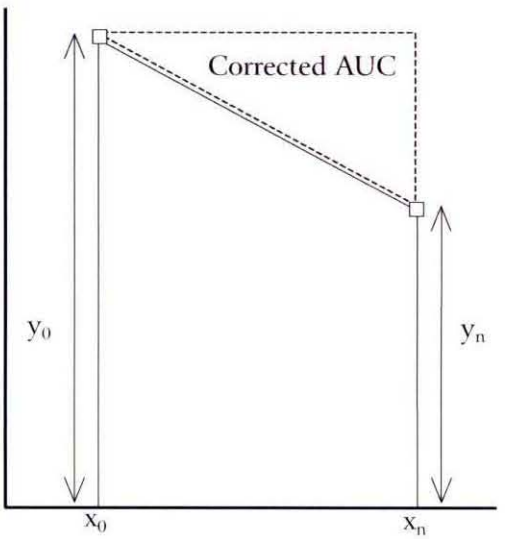

Figure 9. The area of a trapezium between two observations

3.7.3 Maximum glucose response

In addition to the AUC, the absolute change in glucose concentration from the baseline was used to compare the glucose responses between treatments. This maximum response was calculated by deducting the lowest whole-blood glucose concentration from the weighted mean of the four baseline whole-blood glucose observations, in the formula:

$$\text{Maximum Glucose Response} = \text{Baseline Glucose} - \text{Minimum Glucose}$$

4. Sampling protocols

4.1 Basal blood collection

To determine the plasma profile of cows during the basal period, 10 mL blood samples were collected in week 1, 3, 5 and 7 postpartum on two consecutive days, for harvest and storage of plasma (Figure 7). The eight samples were planned for days 7 and 8, 21 and 22, 35 and 36, as well as 49 and 50 postpartum. However, actual sampling occurred on days as close to the designated date as was practical, as presented in Table 3, where the day of collection is given as the weighted mean for all cows (n=10), ± the standard deviation (SD).

Table 3. Days of basal sample collection

Sample	Day Planned	Day Collected ± SD
1	7	7.5 ± 1.18
2	8	8.5 ± 1.18
3	21	21.5 ± 1.18
4	22	22.7 ± 1.12
5	35	35.7 ± 1.34
6	36	36.9 ± 1.27
7	49	49.4 ± 1.35
8	50	50.4 ± 1.35

* All samples were designated numbers in order of collection. A total of 92 samples were collected from each cow during the entire experiment (week 1 to week 12).

4.2 Challenges, clamps and intake restriction

On selected days (Figure 7), cows were exposed to insulin challenges and hyperglycaemic clamps, where all treatment periods followed each other sequentially. The control and rbST period were in week 8 and week 9 respectively, where all cows were on *ad libitum* intake. The feed restriction to 80% of the predicted ME requirement was initiated during week 10, followed by the 80% ME period in week 11 and the combination of recombinant bST and 80% ME treatments in week 12 (80% ME + rbST period). The control period was planned for day 56 postpartum, rbST for day 63, feed restriction for day 70, 80% ME for day 77 and the 80% ME + rbST period for day 84 postpartum. Overlapping of the dates for metabolic tests in the schedule and practical considerations

were taken into account, to allow tests on days as close as possible to the designated date (Table 4).

Table 4. Days of treatment periods and intake restriction

Period	Day Planned	Day Performed ± SD
Control	56	57.5 ± 1.96
rbST	63	64.4 ± 1.84
Intake restriction	70	71.4 ± 1.84
80% ME	77	78.7 ± 1.89
80% ME + rbST	84	85.4 ± 1.96

4.3 The insulin challenge protocol

After the cows had become accustomed to the metabolic house and whole-blood glucose stabilized (at least 60 minutes after entering), three 10 mL blood samples were collected at the time designated minus thirty minutes (t–30), t–20 and t–10 minutes, for the harvest of plasma of the baseline period (Table 5). At the time designated zero (t0), the final 10 mL baseline sample was collected, followed immediately by injection of insulin at 0.1 IU/kg body weight. Small blood samples for analysis of glucose concentration in whole-blood were also collected at t–30, t–20, t–10, t0, t10, t20, t25, t30, t35, t40, t45, t50, t55, t60, t65, t70, t80, t90, t100, t120, t150, t180 and t210 (or until blood glucose concentration stabilized). Blood samples (5mL) for the storage of plasma were simultaneously collected at t30, t60, t90, t120 and t150.

Table 5. Timing of samples in the insulin challenge

Time	10 mL Sample	5 mL Sample	1 mL Sample
t –30	✓		✓
t –20	✓		✓
t –10	✓		✓
t 0	✓		✓
Insulin Injection			
t 10			✓
t 20			✓
t 25			✓
t 30		✓	✓
t 35			✓
t 40			✓
t 45			✓
t 50			✓
t 55			✓
t 60		✓	✓
t 65			✓
t 70			✓
t 80			✓
t 90		✓	✓
t 100			✓
t 120		✓	✓
t 150		✓	✓
t 180			✓
t 210			✓

4.4 The hyperglycaemic clamp protocol

After stabilization of the blood glucose concentration following insulin challenge, another three 10 mL baseline samples (for hyperglycaemic clamp) were collected at the times designated t–30, t–20 and t–10 (Table 6). At the time designated zero (t0), another baseline sample was taken, followed immediately by injection of the 60% w/v glucose solution. Small blood samples for the determination of whole-blood glucose concentration were collected at t–30, t–20, t–10, t0, t5, t10, t15, t20, t25, t30, t40, t50, t60, t70, t80, t90, t100, t110 and t120. Blood samples (10 mL or 5 mL) for plasma storage were collected at t20, t40, t60, t80, t90, t100, t110 and t120.

Table 6. Timing of samples in the hyperglycaemic clamp

Time	10 mL Sample	5 mL Sample	1 mL Sample
t –30	✓		✓
t –20	✓		✓
t –10	✓		✓
t 0	✓		✓
Glucose Injection			
t 5			✓
t 10			✓
t 15			✓
t 20	✓		✓
t 25			✓
t 30			✓
t 40	✓		✓
t 50			✓
t 60	✓		✓
t 70			✓
t 80	✓		✓
t 90		✓	✓
t 100	✓		✓
t 110		✓	✓
t 120	✓		✓

5. Sample analyses

5.1 Basal samples

5.1.1 Plasma glucose concentration

Individual basal samples were analyzed for the concentration of glucose in plasma (mg/dL), utilizing an enzymatic technique (YSI 2300 STAT Plus Glucose and Lactate Analyzer). Each collected plasma sample was analyzed and recorded individually.

The individual variation in plasma glucose (within cow, between the two consecutive samples) in the basal period was generally below 10%, but high in one instance (19.7%). Some variation was to be expected, as these values represent nonfasting plasma glucose concentrations (Table 7). Both the individual plasma glucose concentrations and the

fortnightly weekly average plasma glucose concentrations were recorded. The weighted average of the two consecutive samples was used to calculate the plasma glucose concentration for that week (e.g. 57.6 mg/dL ± 11.1%, week 1).

Table 7. Variation in consecutive basal period plasma glucose samples

Cow	Week 1	Week 3	Week 5	Week 7
620	67.3 ± 5.6%*	66.3 ± 2.6%	66.4 ± 4.7%	62.9 ± 5.6%
515	64.0 ± 10.2%	56.9 ± 1.6%	62.8 ± 1.4%	59.6 ± 2.0%
608	54.2 ± 0.8%	51.7 ± 4.0%	64.1 ± 7.5%	59.6 ± 3.0%
524	58.5 ± 4.1%	57.4 – #	61.8 – #	61.8 ± 0.0%
606	60.7 ± 2.1%	56.5 ± 0.4%	67.6 ± 7.5%	64.2 ± 6.1%
705	51.8 ± 19.7%	61.7 ± 0.6%	58.0 ± 2.0%	59.2 ± 6.6%
631	57.7 ± 2.7%	63.6 ± 0.7%	60.9 ± 0.8%	56.9 ± 4.2%
721	61.3 ± 9.6%	59.2 ± 4.3%	61.8 ± 0.9%	60.5 ± 6.3%
543	55.9 ± 3.8%	62.5 ± 2.5%	64.1 ± 2.0%	62.3 ± 0.1%
521	45.0 ± 9.3%	66.8 ± 1.3%	59.4 ± 7.9%	64.9 ± 5.2%
Mean	**57.6 ± 11.1%**	**60.2 ± 8.0%**	**62.7 ± 4.7%**	**61.2 ± 4.0%**

* All values are presented as a weighted average ± coefficient of variation (CV), or the SD as a percentage of the mean.
\# Samples not available for analysis, due to inability to sample or tube breakage.

5.1.2 Plasma insulin concentration

The basal samples were analyzed using a sandwich enzyme-linked immunosorbent assay (ELISA), developed for the analysis of bovine insulin (DRG, EIA-2340). Variation between the two consecutive samples occurred because the insulin concentration will partly be determined by the time after meal consumption. Individual assay of the fortnightly samples (Table 8) in a single randomly selected cow revealed large variation between the two samples (CV between 8% and 34%), where no bulking of samples occurred. All other basal samples analyses were conducted with bulking, which involved mixing of equal volumes from each sample into a single aliquot, which was assayed as a "single sample", to incorporate the expected variation of the nonfasting plasma insulin concentrations into the fortnightly values.

Due to the nature of the experiment, samples varied greatly in period of storage. Although initial planning clearly stated very little risk, it was later confirmed that storage time did have a significant effect on the insulin concentration (µg/L) in plasma. Although the basal, fortnightly plasma insulin concentrations of all cows were assayed, comparison between the two runs (as well as the samples of varying age on the same plate) could not be done due to unacceptably high variation and variable loss of peptide. The control sample run with the experiment samples in the second run was greatly decreased (at –239% of the first run), with an undetectable insulin concentration. Two additional results of samples previously analyzed revealed inconsistent reductions of –12.2% and –118% of the values obtained in the first assay run and no further use was made of this data.

Table 8. Samples assayed in the basal period

Sample *	SINGLE COW			OTHER COWS		
Number	Glucose	Insulin	IGF-I	Glucose	Insulin	IGF-I
1	✓	✓	✓	✓	①	⑤
2	✓	✓	✓	✓	①	⑤
3	✓	✓	✓	✓	②	⑥
4	✓	✓	✓	✓	②	⑥
5	✓	✓	✓	✓	③	⑦
6	✓	✓	✓	✓	③	⑦
7	✓	✓	✓	✓	④	⑧
8	✓	✓	✓	✓	④	⑧

* Samples were designated numbers in order of collection. Samples that were analyzed individually and not bulked with other samples are designated ✓. Samples that were bulked together are designated by the same circled number, for example ① where sample number 1 and 2 were bulked, mixed and analyzed as "a single sample" for insulin concentration.

5.1.3 Plasma IGF-I concentration

Plasma IGF-I concentrations (µg/L) were analyzed using a sandwich ELISA developed for the analysis of human IGF-I (IDS, AC-27F1). The basal samples of an individual cow were assayed separately, while the samples for other cows were bulked similar to the protocol used for insulin assay (Table 8).

The IGF peptide in plasma is prone to destruction and it became clear that samples could not be stored for long periods of time and should have been assayed shortly after collection. Although we were assured that analysis protocols would be in order, the first run of the assay clearly indicated otherwise. Loss of peptide was already evident and further comparison of samples of varying age was clearly not advisable. Although the full data of four cows was recorded, no IGF-I concentrations were included in the data set.

5.2 The insulin challenge

5.2.1 Whole-blood glucose concentration

Once the cows became used to the conditions of the metabolic house, small whole-blood glucose samples were assayed to establish the stability of the blood glucose concentration. The whole-blood samples were analyzed using the same technique as the plasma assay (YSI), which rapidly made the results available. All the whole-blood samples collected during the insulin challenge (as set out in Table 5) were analyzed for glucose concentration. During the baseline of the control period the average whole-blood glucose concentration within each cow exhibited little variation, as these observations represent the fasting plasma glucose concentrations of the control period. The CV of the control period baseline was 1.5% (range = 0.5% to 3.2%), with an average concentration for all cows of 48.7 mg/dL. Similar results were obtained for variation within cow in the rbST period (CV 1.5%, from 0.4% to 2.4%), the 80% ME period (CV 1.3%, from 0.5% to 2.0%) and the 80% ME + rbST period (CV 1.6%, from 0.5% to 3.8%). There was no significant difference ($P < 0.70$) between the individual baseline observations, therefore the whole-blood AUC was used to compare the effects of treatments on the baseline concentrations.

After insulin injection, frequent whole-blood samples were collected and analyzed for blood glucose concentration. These values were used to plot and monitor blood glucose concentrations during the insulin challenge. The AUC response was calculated by correcting the AUC from t0 to t30 for the baseline AUC and this corrected value was used to compare the glucose responses to insulin injection between treatments.

5.2.2 Plasma glucose concentration

Each individual baseline plasma sample collected was analyzed for the concentration of glucose in mg/dL for all cows in the control period (Table 9). As with the whole-blood glucose concentrations, there was very little variation between the four baseline samples for the insulin challenge (samples 9 to 12), which were not significantly different ($P < 0.14$). The average CV within cows for the four time points was 1.8% with a range between 0.5 to 3.5% in the control period. Similar results were obtained for the rbST period (CV 1.8%, range = 0.8% to 3.0%), the 80% ME period (CV 2.1%, range = 0.9% to 2.8%) and the 80% ME + rbST period (CV 2.1%, range = 0.6% to 4.2%). For the other treatment periods, each baseline plasma sample was separately analyzed for glucose concentration (Table 10). The individual plasma glucose concentrations and the plasma AUC from t–30 to t0 were used to determine the effects of treatments.

All the plasma samples of the insulin response were likewise analyzed for the plasma glucose concentration, which included samples 13 to 17 in Table 9 and Table 10. The corrected plasma AUC was used to determine the glucose response to insulin injection of treatments.

Table 9. Samples assayed in the control period (insulin challenge)

		SINGLE COW			OTHER COWS		
Sample *	Time	Glucose	Insulin	IGF-I	Glucose	Insulin	IGF-I
9	–30	✓	✓	✓	✓	×	①
10	–20	✓	✓	✓	✓	×	①
11	–10	✓	✓	✓	✓	×	①
12	0	✓	✓	✓	✓	×	①
13	30	✓	×	✓	✓	×	×
14	60	✓	×	×	✓	×	×
15	90	✓	×	✓	✓	×	×
16	120	✓	×	×	✓	×	×
17	150	✓	×	×	✓	×	×

* Samples 9 - 12 refer to the baseline period prior to insulin challenge, with samples 13 - 17 obtained between t30 and t150 minutes after insulin injection. Samples that were individually analyzed are designated ✓, with samples not analyzed designated ×. Samples that were bulked are indicated with the same circled number.

5.2.3 Plasma insulin concentration

The individual plasma samples for a single cow were analyzed for insulin concentration during the initial baseline of the control period (Table 9). For other cows, no samples were assayed for the insulin challenge in any of the treatment periods (Table 9 and Table 10). The insulin concentration during the insulin challenge was not included in the data set, for any of the cows sampled (see paragraph 5.1.2).

5.2.4 Plasma IGF-I concentration

The individual baseline plasma samples from the control period (fasting IGF-I concentration), as well as two samples during the response phase were analyzed in a single cow (Table 9). In the other treatment periods only the fasting plasma IGF-I concentrations were determined by bulking the individual plasma samples (Table 10). No IGF-I values were used during the statistical analysis of the data after completion of only a single assay run (see paragraph 5.1.3).

Table 10. Samples assayed in rbST and/or 80% ME periods (insulin challenge)

		SINGLE COW			OTHER COWS		
Samples	Time	Glucose	Insulin	IGF-I	Glucose	Insulin	IGF-I
30, 51, 72	–30	✓	①	②	✓	×	③
31, 52, 73	–20	✓	①	②	✓	×	③
32, 53, 74	–10	✓	①	②	✓	×	③
33, 54, 75	0	✓	①	②	✓	×	③
34, 55, 76	30	✓	×	×	✓	×	×
35, 56, 77	60	✓	×	×	✓	×	×
36, 57, 78	90	✓	×	×	✓	×	×
37, 58, 79	120	✓	×	×	✓	×	×
38, 59, 80	150	✓	×	×	✓	×	×

* The baseline samples for insulin challenge include samples 30 - 33 (rbST), 51 - 54 (80% ME) and 72 - 75 (80% ME + rbST). The symbol ✓ indicates samples assayed individually, × indicates samples not assayed, while bulked samples are represented by a circled number.

5.3 The hyperglycaemic clamp

5.3.1 Whole-blood glucose concentration

Once the whole-blood glucose concentration stabilized after the insulin challenge, the hyperglycaemic clamp was initiated. All the 1 mL whole-blood samples collected during the hyperglycaemic clamp (as set out in Table 6) were immediately analyzed and plotted during the clamp for the control period (Table 12) and treatment periods (Table 13). Once the glucose infusion commenced, whole-blood glucose concentrations were plotted and monitored to maintain hyperglycaemia. The average whole-blood glucose concentration was 47.5 mg/dL in the control period, with little individual variation between the four baseline observations. The CV was 2.0%, with a range between 1.0 to 3.1% in the control period, with similarly low values in the rbST period (CV 1.7%, range from 0.3% to 3.1%), the 80% ME period (CV 1.6%, range 0.4% to 3.4%) and the 80% ME + rbST period (CV 1.8%, range 0.5% to 3.5%). Although there was an effect of the time of sampling (t–30, t–20, t–10 and t0), this was generally very small. This included significant ($P < 0.05$) differences for 7 of the 24 observations, which were related to differences of between 1.8% and 2.4% of the average baseline glucose concentration (Table 11). Only the whole-blood glucose concentrations were used in the data set.

Table 11. Variation in individual baseline samples (hyperglycaemic clamp)

Time Points	*Control*	*rbST*	*80% ME*	*80% ME + rbST*
t–30 *vs.* t–20	–	–	–	–
t–30 *vs.* t–10	1.8%	–	–	1.8%
t–30 *vs.* t0	2.4%	2.0%	2.0%	–
t–20 *vs.* t–10	–	–	–	–
t–20 *vs.* t0	1.9%	–	–	2.4%
t–10 *vs.* t0	–	–	–	–
Average	**47.5 mg/dL**	**41.5 mg/dL**	**45.5 mg/dL**	**44.5 mg/dL**

During the final 40 minutes of hyperglycaemia the system stabilized, as confirmed by the stable GIR of this phase. There was no difference between the individual GIR values of t90, t100, t110 and t120 ($P < 0.16$) and this represented a steady-state (SSGIR). The average concentration increment of the plateau phase was +48.3 mg/dL for the control period, +51.7 mg/dL for the rbST period, +49.4 mg/dL for the 80% ME period and +52.6 mg/dL for the 80% ME + rbST period. The target level of hyperglycaemia was +50.0 mg/dL (± 10%) and was achieved in every instance (i.e. for all cows in all clamps).

5.3.2 Plasma glucose concentration

Each individual baseline plasma sample collected during a hyperglycaemic clamp was analyzed for all cows in the control and treatment periods (Table 12 and Table 13 respectively). The four baseline samples from the control period exhibited little variation with a CV within cows of 1.6% of the 65.0 mg/dL (range of 0.5% to 3.0%), with similar values for the rbST period (CV 3.3%, range of 1.5% to 4.7%), 80% ME period (CV 3.3%, range of 1.0% to 5.2%) and the 80% ME + rbST (CV 2.4%, range of 1.6% to 3.9%). There was no difference between the glucose concentrations of the four baseline samples ($P < 0.52$). The individual concentrations were therefore used to calculate the weighted mean concentration, which was used to compare the baseline values for the hyperglycaemic clamp between treatments.

From t90 to t120 of the hyperglycaemic clamp (the plateau phase) the average plasma glucose concentration increased from the baseline (65.0 mg/dL) to plateau (131.4 mg/dL) was +66.4 mg/dL for the control period. The plasma glucose increment was +71.2 mg/dL for the rbST period, +66.9 mg/dL for the 80% ME period and +70.2 mg/dL for the 80% ME + rbST period.

Table 12. Samples assayed in the control period (glucose clamp)

		SINGLE COW			OTHER COWS		
Sample *	Time	Glucose	Insulin	IGF-I	Glucose	Insulin	IGF-I
18	–30	✓	✓	✓	✓	①	③
19	–20	✓	✓	✓	✓	①	③
20	–10	✓	✓	✓	✓	①	③
21	0	✓	✓	✓	✓	①	③
22	20	✓	×	×	✓	×	×
23	40	✓	✓	✓	✓	×	×
24	60	✓	×	×	✓	×	×
25	80	✓	×	×	✓	×	×
26	90	✓	✓	✓	✓	②	④
27	100	✓	✓	✓	✓	②	④
28	110	✓	✓	✓	✓	②	④
29	120	✓	✓	✓	✓	②	④

*The hyperglycaemic clamp baseline samples included samples 18 - 21 for the control period. Hyperglycaemia was reached and clamped at +50 mg/dL by 30 minutes, or before sample 23. The plateau period encompassed samples 26 - 29. Individual samples that were analyzed are designated ✓ and those not analyzed as ×. Samples that were bulked together have the same circled number.

5.3.3 Plasma insulin concentration

For a single cow, the baseline samples and plateau samples (during SSGIR) of the control period was analyzed separately (Table 12), together with one sample of the rising phase of circulating blood glucose concentration. For all other cows and in the other treatment periods (Table 13) samples collected during the baseline and plateau phases were bulked. The insulin increment between baseline and plateau (or MPII) estimates the degree of reaction of the pancreas to a supraphysiological glucose concentration, where gluconeogenesis should not contribute to the glucose pool in blood. Due to the conditions described in paragraph 5.1.2, no insulin values were included in the data set.

5.3.4 Plasma IGF-I concentration

For a single cow, the baseline and plateau samples during the control period were analyzed separately (Table 12), while a single sample between the baseline and plateau was also assayed. For the treatment periods all the samples assayed were bulked for the baseline and plateau phases (Table 13). No further assays of plasma IGF-I samples were conducted (see paragraph 5.1.3).

Table 13. Samples assayed in rbST and/or 80% ME periods (glucose clamp)

		SINGLE COW			OTHER COWS		
Samples	Time	Glucose	Insulin	IGF-I	Glucose	Insulin	IGF-I
39, 60, 81	–30	✓	①	③	✓	⑤	⑦
40, 61, 82	–20	✓	①	③	✓	⑤	⑦
41, 62, 83	–10	✓	①	③	✓	⑤	⑦
42, 63, 84	0	✓	①	③	✓	⑤	⑦
43, 64, 85	20	✓	×	×	✓	×	×
44, 65, 86	40	✓	×	×	✓	×	×
45, 66, 87	60	✓	×	×	✓	×	×
46, 67, 88	80	✓	×	×	✓	×	×
47, 68, 89	90	✓	②	④	✓	⑥	⑧
48, 69, 90	100	✓	②	④	✓	⑥	⑧
49, 70, 91	110	✓	②	④	✓	⑥	⑧
50, 71, 92	120	✓	②	④	✓	⑥	⑧

* Baseline samples include samples 39 - 42 (rbST), 60 - 63 (80% ME) and 81 - 84 (80% ME + rbST). The plateau period samples include samples 47 - 50 (rbST), 68 - 71 (80% ME) and 89 - 92 (80% ME + rbST). Samples analyzed separately (✓), not analyzed (×) and bulked (circled numbers) are indicated.

6. Sample assay techniques

6.1 Glucose concentration

The glucose concentration in whole-blood and plasma was determined by a method utilizing an enzymatic technique that determined the concentration of glucose in samples against a known standard of 180 mg/dL (YSI 2300 STAT Plus). Briefly, glucose diffuses across a 3-layered immobilized membrane (Figure 10) that contains enzyme, *glucose oxidase* and a cellulose acetate layer that protects a platinum anode from other oxidizing substrates and contamination (YSI, 1997). Enzymatic glucose oxidation results in hydrogen peroxide (H_2O_2) production, which undergoes non-enzymatic oxidation at the platinum anode, resulting in electron ($e-$) production. The glucose concentration is proportional to the steady-state hydrogen peroxide concentration, which in turn is proportional to $e-$ flow. The difference in $e-$ flow in nanoampere (nA) resulting from the known calibration standard diluted into 600 µL buffer (steady-state plateau current – baseline current) is used to determine the glucose concentration in controls or samples. These results were automatically corrected for temperature differences.

Because of the instability of glucose concentration in whole-blood, control samples were not available for the assays of glucose concentration in whole-blood. During the analysis of plasma, two control samples that were collected and frozen with the experiment samples were used as controls. The average concentration of control 1 was 48.8 mg/dL with an average SD calculated within each run, of 0.50 mg/dL (or 1.0%, range = 0.0% to 3.3%). The difference between runs was also small, with a between-assay SD of 0.57 m/dL, or 1.16%. The average concentration of control 2 was 52.0 mg/dL, with a within-assay SD of 0.62 mg/dL (or 1.2%, range = 0.1% to 3.4%) and between-assay SD of 0.59 mg/dL (or 1.1%).

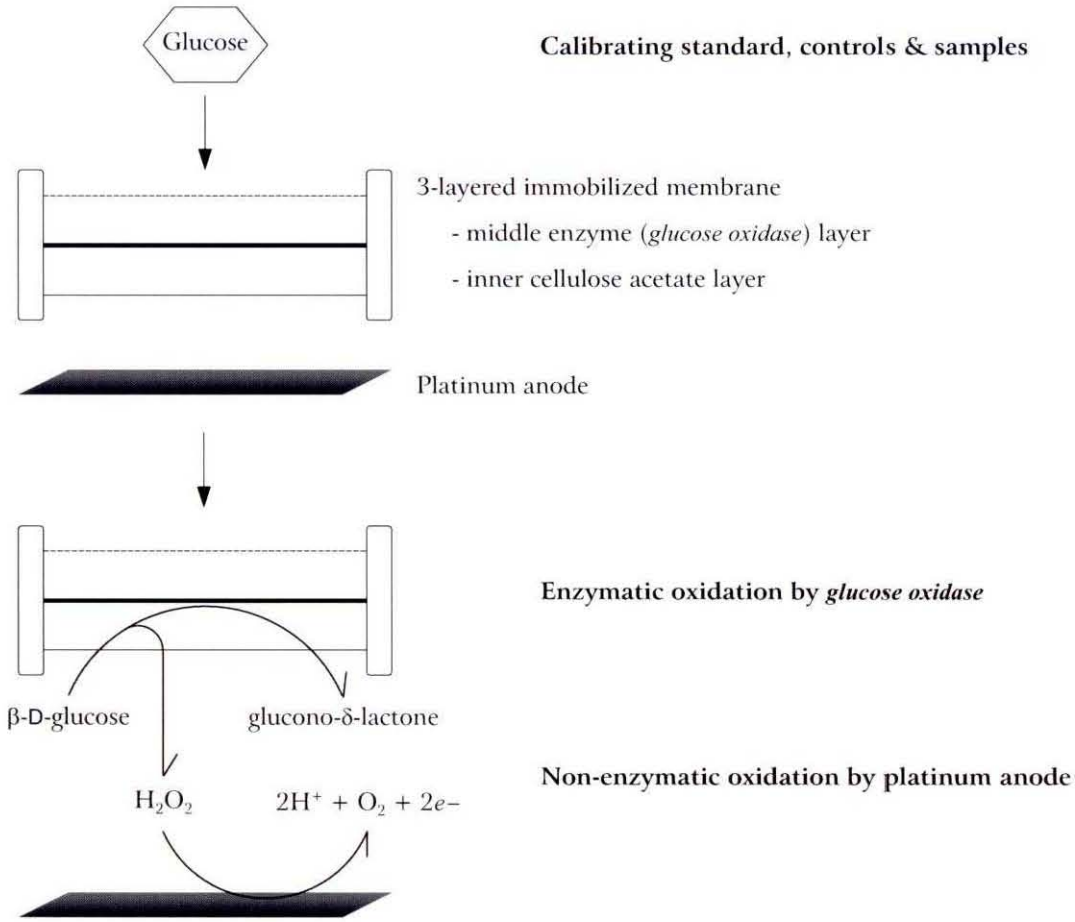

Figure 10. Diagrammatic representation of glucose assay (YSI, 1997)

6.2 Plasma insulin concentration

The plasma insulin concentration was determined using a bovine insulin ELISA kit (DRG, EIA-2340). This ELISA technique (DRG, 2001) utilized solid phase, 2-site binding of the insulin in the samples with tetramethylbenzidine (TMB) as a chromogenic substrate (Figure 11). The kit supplied a 96 well plate, with 7 standards (0, 0.25, 1.0, 2.0, 4.0 and 6 μg/L) run in duplicate to generate the standard curve from which sample concentrations were calculated. A control sample collected and frozen during the experiment was also included in the assays.

The control, together with the standards, allowed for the analysis of 80 samples per kit. During the first run, a total of 21 samples were analyzed separately for a single cow, with 59 more observations as bulked samples. The total number of plasma samples, regardless of bulking, that were used during the first run was 225 that included samples from 6 cows (5 complete analyses + 1 partial). During the second run, 70 bulked samples were analyzed, which included 232 samples from 6 cows (5 complete analyses + 1 partial). The insulin concentration analysis was therefore completed in two runs, utilizing 457 samples. Due to peptide instability with long-term storage (decay of control and samples), insulin data was not included in the statistical analyses (paragraph 5.1.2).

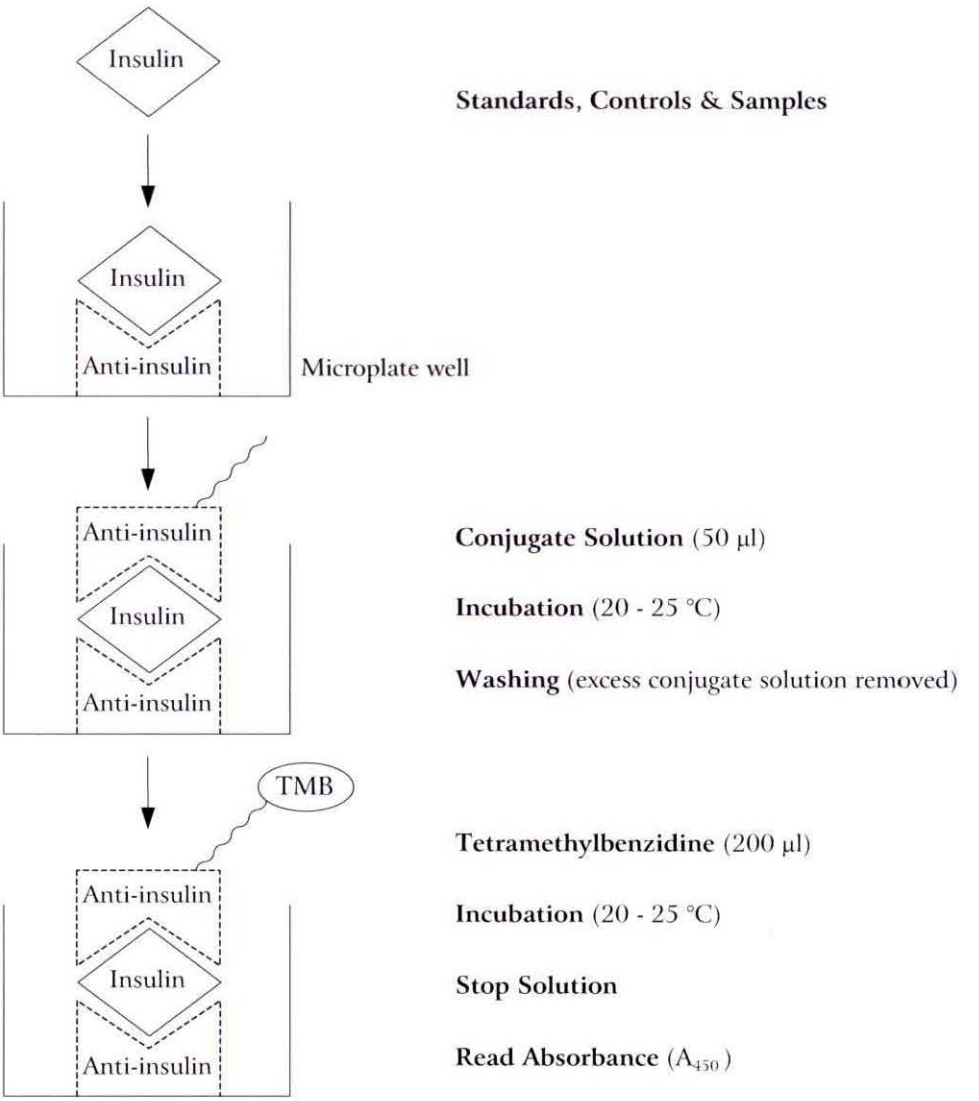

Figure 11. Diagrammatic representation of the insulin ELISA (DRG, 2001)

6.3 Plasma IGF-I concentration

Plasma IGF-I concentration was determined using an IGF-I ELISA kit (IDS, AC-27F1). The technique (IDS, 2001) utilized solid phase, 2-site binding of the total IGF-I in the samples, after release from binding proteins (Figure 12). The technique allows for the analysis of 80 samples, once the 6 standards used to generate a standard curve (0, 15, 34, 119, 360 and 1201 µg/L), a control supplied with the kit and an own control have been added to the 96 well plate.

During the first run, a total of 23 samples were analyzed separately for a single cow, with 57 more observations as bulked samples. The total number of plasma samples, regardless of bulking, that were used during this assay was 227, which included samples from 4 cows. Due to peptide decay in samples and controls and the varying age of stored plasma samples, no IGF-I data was included in the data set (paragraph 1.5.3).

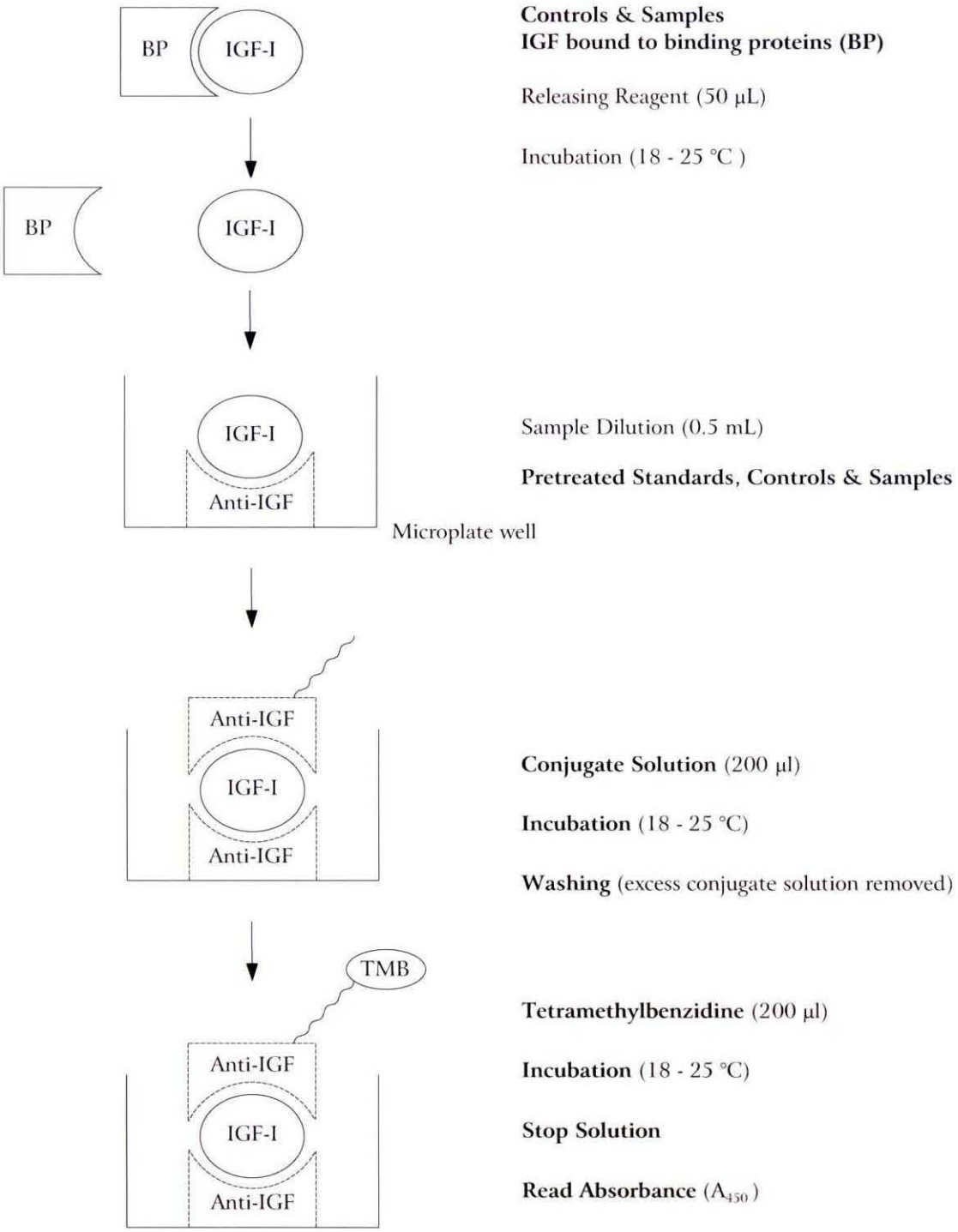

Figure 12. Diagrammatic representation of the IGF-I ELISA (IDS, 2001)

CHAPTER 4. DATA AND STATISTICAL ANALYSES

1. Production data

1.1 Body weight

The body weight in kilograms determined weekly on two consecutive days was used to calculate a weighted average of two observations. The results of the average body weight for each week of the experiment are presented in Table 14 and Figure 13 (n=10) as weekly, weighted average body weights. All results are given as the mean of all cows ± standard deviation (SD), with the SD as a percentage of the mean, or coefficient of variation (CV).

Table 14. Weekly mean body weight

Week	Mean ± SD	CV
1	612.0 ± 59.3 kg	9.7%
2	602.8 ± 58.9 kg	9.8%
3	594.3 ± 59.4 kg	10.0%
4	589.3 ± 59.7 kg	10.1%
5	589.5 ± 62.8 kg	10.7%
6	582.8 ± 66.3 kg	11.4%
7	587.5 ± 65.4 kg	11.1%
8	580.8 ± 61.4 kg	10.6%
9	576.3 ± 57.3 kg	9.9%
10	577.8 ± 55.0 kg	9.5%
11	562.8 ± 53.4 kg	9.5%
12	558.6 ± 60.1 kg	10.8%

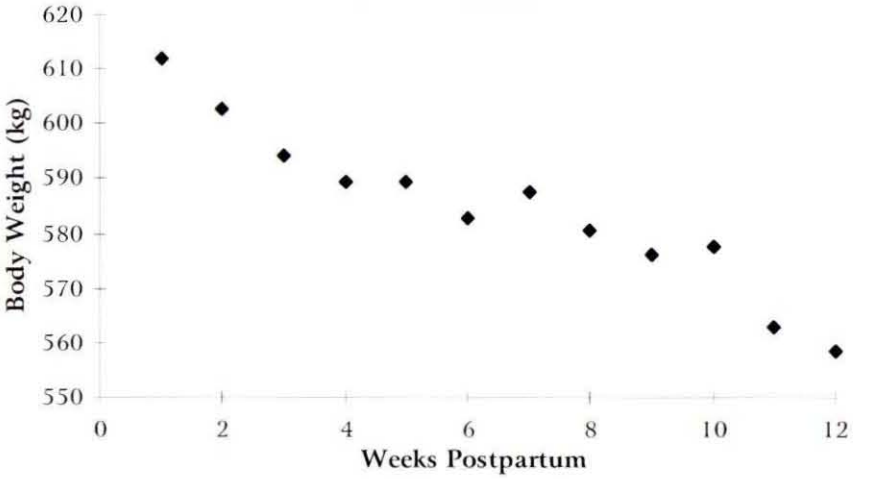

Figure 13. Weekly mean body weight

1.2 Body condition score

The average BCS of two observations, from two consecutive days was determined (1=emaciated, 5=obese) for each cow. The average BCS by week postpartum is given in Table 15 and Figure 14.

Table 15. Weekly mean BCS

Week	Mean ± SD	CV
1	3.2 ± 0.2	5.9%
2	3.2 ± 0.2	6.5%
3	3.1 ± 0.2	6.5%
4	3.1 ± 0.2	7.8%
5	3.1 ± 0.2	7.7%
6	3.0 ± 0.3	8.3%
7	3.0 ± 0.3	8.3%
8	3.0 ± 0.3	9.2%
9	2.9 ± 0.3	8.6%
10	2.9 ± 0.3	8.5%
11	2.9 ± 0.2	8.3%
12	2.9 ± 0.2	8.3%

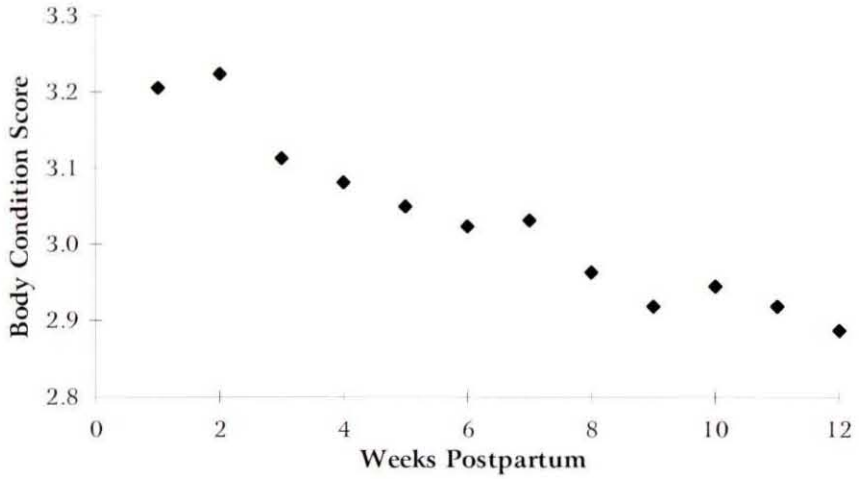

Figure 14. Weekly mean body condition score

1.3 Milk production

The milk production was determined for the morning and afternoon milkings. The weighted average for each week was determined from these daily milk production records (Table 16 and Figure 15).

Table 16. Weekly mean milk production

Week	Mean ± SD	CV
1	20.4 ± 4.0 kg/d	19.8%
2	28.2 ± 5.3 kg/d	18.9%
3	29.6 ± 5.4 kg/d	18.2%
4	29.5 ± 5.5 kg/d	18.5%
5	30.0 ± 5.6 kg/d	18.8%
6	30.1 ± 5.3 kg/d	17.6%
7	29.5 ± 4.5 kg/d	15.4%
8	28.6 ± 5.2 kg/d	18.3%
9	28.2 ± 5.2 kg/d	18.6%
10	27.9 ± 6.1 kg/d	21.7%
11	28.2 ± 6.6 kg/d	23.4%
12	26.2 ± 5.3 kg/d	20.4%

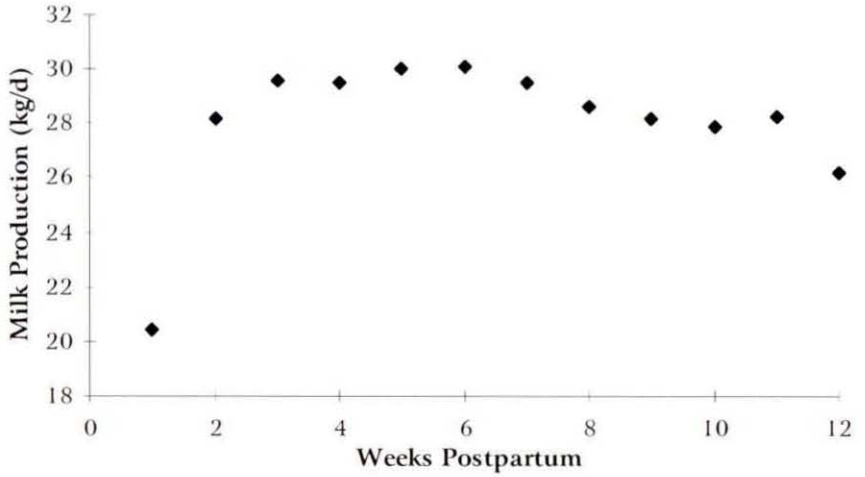

Figure 15. Weekly mean milk production

During the experimental period the mean milk production of the 3 days prior to metabolic test (Figure 16) days exhibited a decline, especially during the periods of energy restriction.

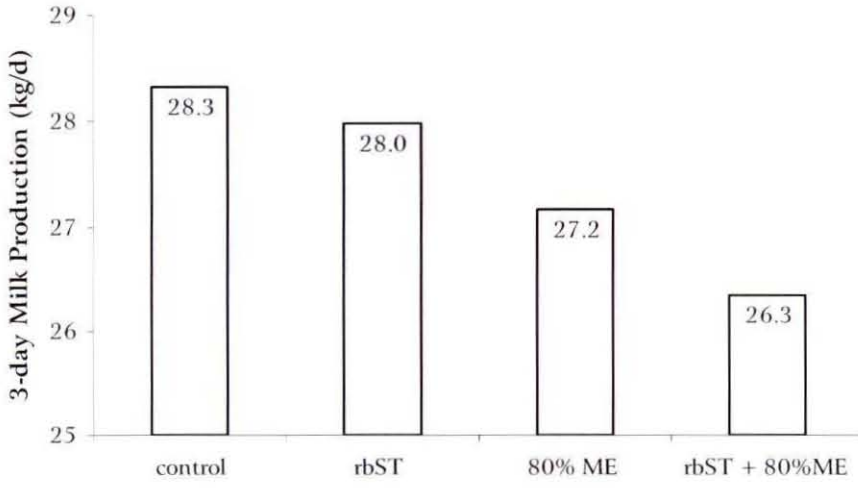

Figure 16. Mean milk production prior to metabolic tests

2. Whole-blood data

2.1 Data collected during the insulin challenge

2.1.1 The control period

The glucose concentration in whole-blood was periodically determined from 30 minutes before insulin injection (t–30) up to 180 minutes after insulin injection (t180). These values are presented as a weighted average for all cows (n=10), at each time point ± SD and CV (Table 17 and Figure 17).

Table 17. Insulin challenge whole-blood glucose concentration (control)

Time	Mean ± SD	CV
t –30	48.5 ± 2.8 mg/dL	5.7%
t –20	49.0 ± 3.1 mg/dL	6.3%
t –10	48.8 ± 3.3 mg/dL	6.7%
t 0	48.6 ± 3.2 mg/dL	6.7%
t 10	44.2 ± 4.0 mg/dL	9.1%
t 20	37.6 ± 3.0 mg/dL	8.0%
t 25	35.4 ± 3.5 mg/dL	9.9%
t 30	33.7 ± 3.6 mg/dL	10.6%
t 35	32.0 ± 3.7 mg/dL	11.5%
t 40	31.8 ± 2.7 mg/dL	8.6%
t 45	32.8 ± 2.5 mg/dL	7.5%
t 50	34.2 ± 2.5 mg/dL	7.4%
t 55	35.8 ± 3.2 mg/dL	9.1%
t 60	36.8 ± 3.5 mg/dL	9.5%
t 65	37.9 ± 3.9 mg/dL	10.3%
t 70	38.6 ± 4.3 mg/dL	11.1%
t 80	40.3 ± 3.8 mg/dL	9.3%
t 90	41.8 ± 3.4 mg/dL	8.2%
t 100	42.6 ± 4.1 mg/dL	9.5%
t 120	44.0 ± 3.9 mg/dL	8.8%
t 150	46.2 ± 3.8 mg/dL	8.2%
t 180	47.8 ± 4.4 mg/dL	9.2%

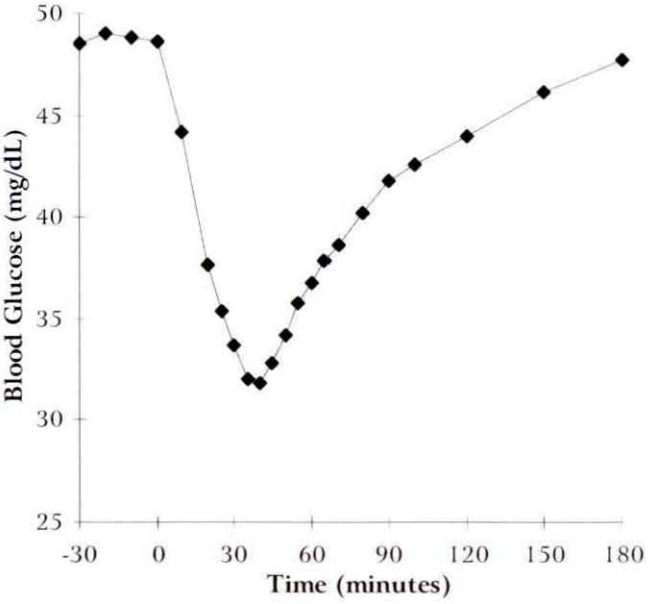

Figure 17. Insulin challenge whole-blood concentration (control)

2.1.2 The rbST period

Whole-blood glucose concentration data collected ± 7 days after recombinant bST treatment is presented in Table 18 and Figure 18. Individual values were used to calculate the weighted average for all cows.

Table 18. Insulin challenge whole-blood glucose concentration (rbST)

Time	Mean ± SD	CV
t –30	43.9 ± 3.4 mg/dL	7.8%
t –20	44.3 ± 3.8 mg/dL	8.5%
t –10	44.0 ± 4.2 mg/dL	9.6%
t 0	44.4 ± 4.2 mg/dL	9.6%
t 10	40.8 ± 3.1 mg/dL	7.5%
t 20	36.0 ± 2.8 mg/dL	7.9%
t 25	33.9 ± 2.3 mg/dL	6.8%
t 30	32.7 ± 2.5 mg/dL	7.6%
t 35	31.5 ± 2.7 mg/dL	8.7%
t 40	30.8 ± 2.3 mg/dL	7.4%
t 45	30.5 ± 2.6 mg/dL	8.5%
t 50	31.2 ± 3.6 mg/dL	11.4%
t 55	32.6 ± 4.3 mg/dL	13.2%
t 60	33.0 ± 4.8 mg/dL	14.5%
t 65	33.8 ± 4.8 mg/dL	14.3%
t 70	34.7 ± 4.9 mg/dL	14.2%
t 80	36.1 ± 4.8 mg/dL	13.3%
t 90	36.9 ± 4.8 mg/dL	13.1%
t 100	37.8 ± 4.5 mg/dL	11.8%
t 120	39.7 ± 4.8 mg/dL	12.0%
t 150	40.7 ± 4.5 mg/dL	11.2%
t 180	41.5 ± 4.8 mg/dL	11.6%

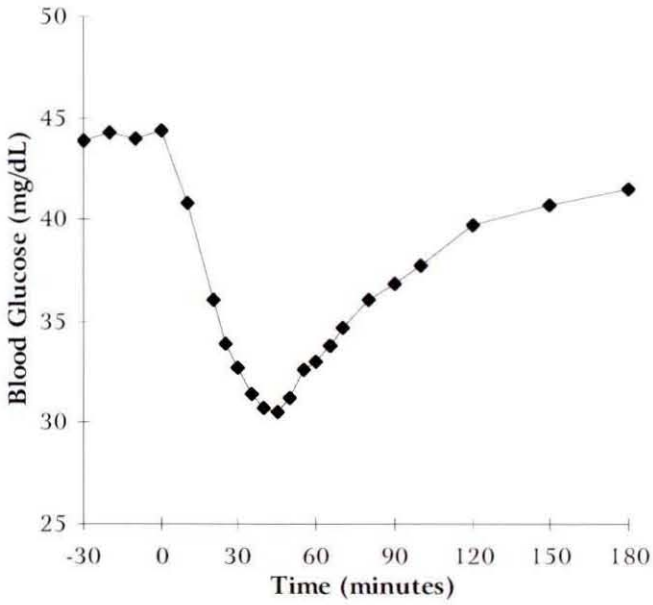

Figure 18. Insulin challenge whole-blood concentration (rbST)

2.1.3 The 80% ME period

The average whole-blood glucose concentration results of the insulin challenge, performed 1 week after energy restriction are presented in Table 19 and Figure 19.

Table 19. Insulin challenge whole-blood glucose concentration (80% ME)

Time	Mean ± SD	CV
t –30	45.8 ± 4.3 mg/dL	9.3%
t –20	45.8 ± 4.4 mg/dL	9.5%
t –10	45.9 ± 3.7 mg/dL	8.2%
t 0	45.5 ± 3.9 mg/dL	8.5%
t 10	42.1 ± 3.5 mg/dL	8.4%
t 20	35.2 ± 3.1 mg/dL	8.7%
t 25	32.9 ± 3.5 mg/dL	10.8%
t 30	30.9 ± 3.9 mg/dL	12.5%
t 35	29.3 ± 3.9 mg/dL	13.4%
t 40	29.2 ± 2.9 mg/dL	9.9%
t 45	30.1 ± 2.8 mg/dL	9.2%
t 50	30.9 ± 2.7 mg/dL	8.7%
t 55	32.7 ± 3.2 mg/dL	9.8%
t 60	33.5 ± 3.9 mg/dL	11.7%
t 65	34.9 ± 4.4 mg/dL	12.7%
t 70	35.8 ± 4.4 mg/dL	12.3%
t 80	38.2 ± 4.1 mg/dL	10.8%
t 90	39.7 ± 4.3 mg/dL	10.9%
t 100	40.9 ± 4.8 mg/dL	11.7%
t 120	42.9 ± 4.1 mg/dL	9.7%
t 150	43.1 ± 3.5 mg/dL	8.2%
t 180	44.9 ± 3.8 mg/dL	8.5%

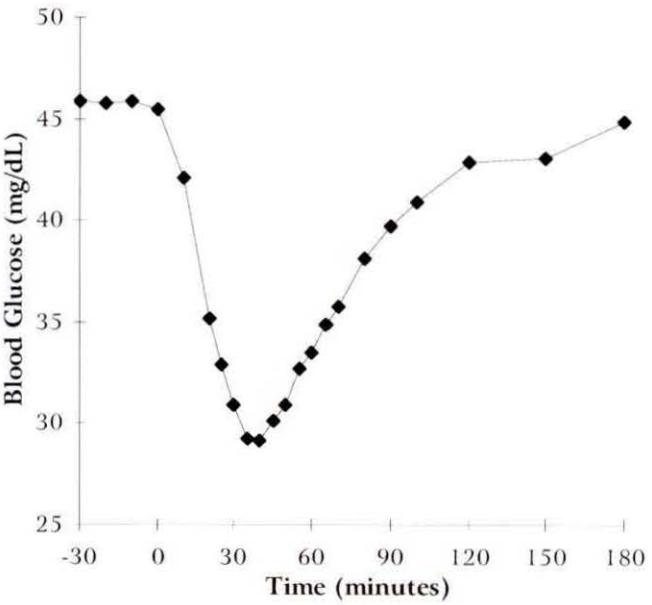

Figure 19. Insulin challenge whole-blood concentration (80% ME)

2.1.4 The 80% ME + rbST period

Whole-blood data collected periodically during the insulin challenge performed to test the combined effects of 80% of metabolizable energy and recombinant bST treatments, is presented in Table 20 and Figure 20.

Table 20. Insulin challenge whole-blood glucose concentration (80% ME + rbST)

Time	Mean ± SD	CV
t –30	46.7 ± 2.9 mg/dL	6.2%
t –20	46.6 ± 2.6 mg/dL	5.6%
t –10	46.6 ± 2.6 mg/dL	5.6%
t 0	46.4 ± 3.4 mg/dL	7.3%
t 10	43.5 ± 3.2 mg/dL	7.3%
t 20	37.5 ± 2.9 mg/dL	7.7%
t 25	35.2 ± 3.0 mg/dL	8.5%
t 30	33.3 ± 2.6 mg/dL	7.8%
t 35	31.5 ± 2.7 mg/dL	8.5%
t 40	30.7 ± 2.6 mg/dL	8.4%
t 45	31.1 ± 3.1 mg/dL	10.1%
t 50	32.1 ± 4.2 mg/dL	12.9%
t 55	33.1 ± 4.7 mg/dL	14.3%
t 60	33.8 ± 4.6 mg/dL	13.7%
t 65	34.8 ± 5.2 mg/dL	14.8%
t 70	36.7 ± 5.2 mg/dL	14.2%
t 80	38.1 ± 5.1 mg/dL	13.4%
t 90	39.3 ± 3.9 mg/dL	9.9%
t 100	40.8 ± 3.7 mg/dL	9.1%
t 120	42.3 ± 3.2 mg/dL	7.7%
t 150	43.5 ± 2.6 mg/dL	5.9%
t 180	44.6 ± 3.2 mg/dL	7.1%

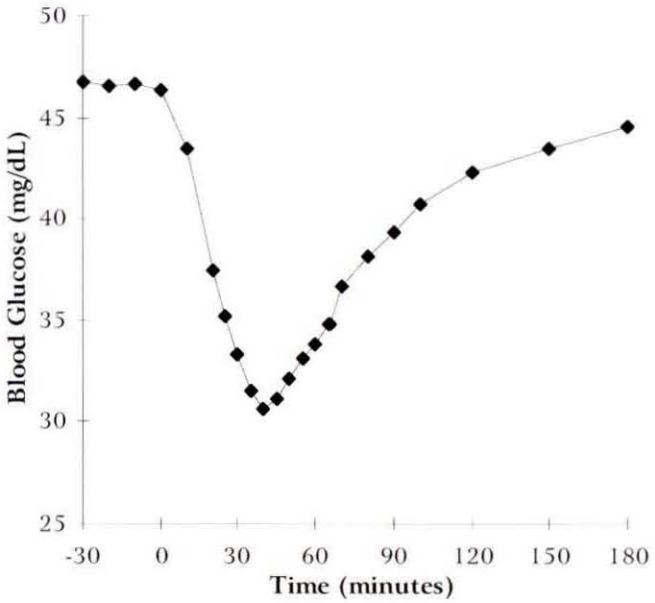

Figure 20. Insulin challenge whole-blood concentration (80% ME + rbST)

2.2 Effect of treatments in the insulin challenge

2.2.1 The baseline glucose concentration

The individual baseline glucose concentrations determined at 10-minute intervals from t–30 to t0 were analyzed using repeated measures between periods (SAS, 1994). There was no significant effect of time of sampling on glucose concentration ($P < 0.7005$), but there was a significant effect ($P < 0.0001$) of period on the individual baseline measurements (Table 21 and Figure 21).

Table 21. The insulin challenge baseline whole-blood concentration

Period	Description	Mean ± SD	CV
1	Control	[a] 48.7 ± 3.0 mg/dL	6.1%
2	rbST	[b] 44.1 ± 3.8 mg/dL	8.6%
3	80% ME	[c] 45.7 ± 3.9 mg/dL	8.6%
4	80% ME + rbST	[d] 46.6 ± 2.8 mg/dL	6.0%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

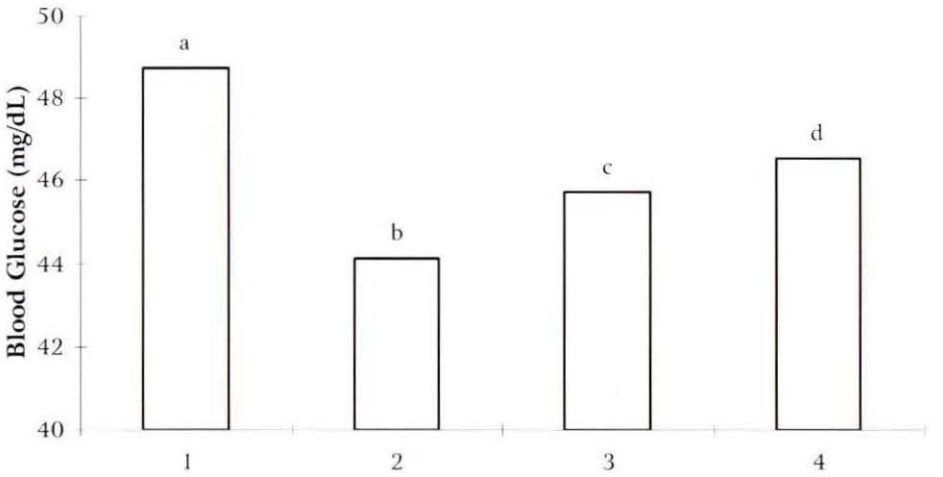

Figure 21. The insulin challenge baseline (whole-blood)

2.2.2 The baseline glucose AUC

The area under the successive glucose concentration values was calculated by summation of the areas of the trapezoids under the baseline curve (AUC), between t–30 to t0 (paragraph 3.7.2, Materials and methods). Period significantly ($P < 0.0006$) affected the insulin challenge baseline AUC and the values are presented in Table 22 and Figure 22 as the average for all cows (n=10), during each period. The whole-blood glucose AUC of the 80% ME + rbST period tended to be different from the control ($P < 0.0679$) and the rbST ($P < 0.0571$) periods.

Table 22. The insulin challenge baseline whole-blood AUC

Period	Description	Mean ± SD	CV
1	Control	^{a} 1464.2 ± 91.6 mg × min	6.3%
2	rbST	^{b} 1324.4 ± 117.4 mg × min	8.9%
3	80% ME	^{b} 1373.0 ± 120.9 mg × min	8.8%
4	80% ME + rbST	^{ab} 1397.6 ± 81.9 mg × min	5.9%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

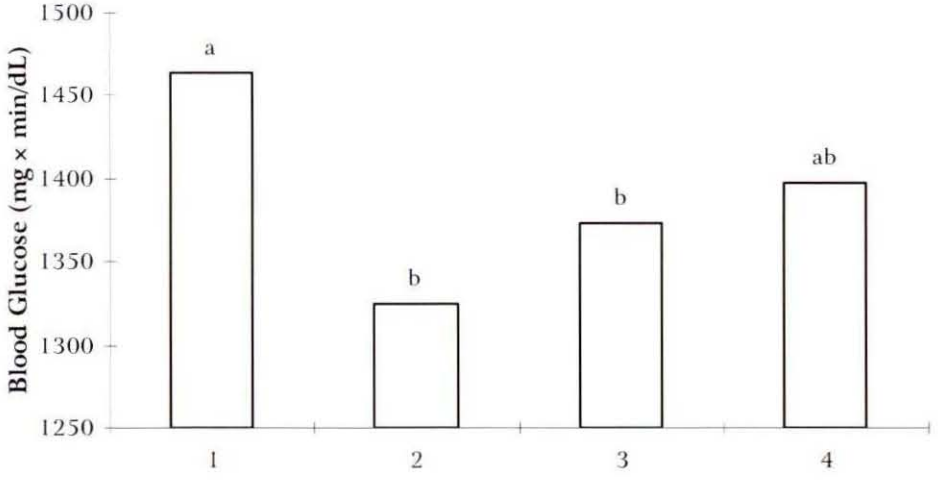

Figure 22. The insulin challenge baseline AUC (whole-blood)

2.2.3 The glucose AUC response to insulin injection (t0 - t30)

The response AUC from t0 to t30 was calculated by summation of the areas of the trapezoids under the response curve, corrected for the baseline AUC. The corrected AUC values are presented in Table 23 and Figure 23. Significant differences between periods were observed ($P < 0.0228$) and differences tended to reach significance between the rbST and 80% ME period ($P < 0.0794$) as well as the control and 80% ME + rbST period ($P < 0.0600$).

Table 23. The insulin challenge whole-blood response AUC

Period	Description	Mean ± SD	CV
1	Control	^{a} −235.7 ± 54.8 mg × min /dL	23.2%
2	rbST	^{b} −172.8 ± 45.8 mg × min /dL	26.5%
3	80% ME	^{ab} −218.9 ± 62.4 mg × min /dL	28.5%
4	80% ME + rbST	^{ab} −190.3 ± 28.1 mg × min /dL	14.8%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

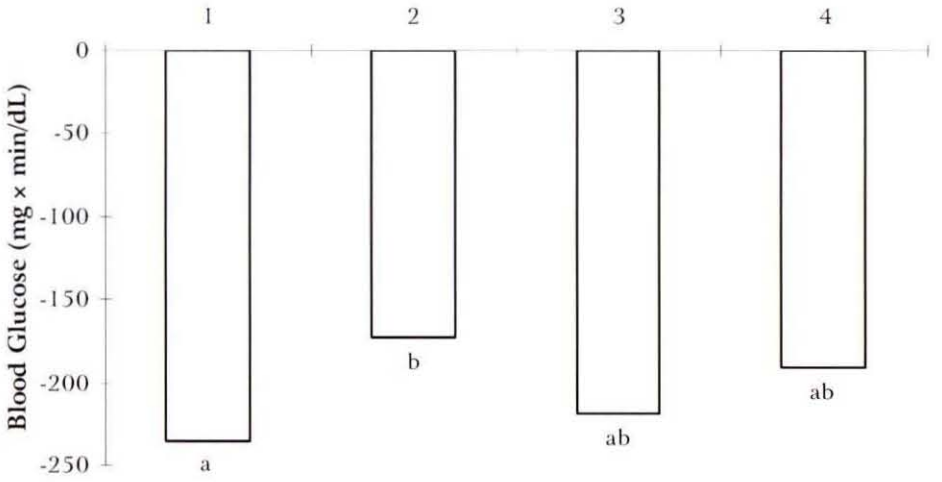

Figure 23. The insulin challenge response AUC (whole-blood)

2.2.4 The maximum glucose response

The maximum glucose response was determined by deducting the minimum glucose concentration recorded during each insulin challenge, from the average baseline glucose concentration for each challenge. There were statistically significant differences ($P < 0.0148$) in the glucose response between periods (Table 24 and Figure 24). Additionally, there was a tendency ($P < 0.0646$) for the 80% ME + rbST period to be different from the rbST period.

Table 24. The insulin challenge maximum response

Period	Description	Mean ± SD	CV
1	Control	^{a} 18.3 ± 3.3 mg/dL	18.2%
2	rbST	^{b} 14.3 ± 3.5 mg/dL	24.4%
3	80% ME	^{a} 17.9 ± 4.5 mg/dL	25.4%
4	80% ME + rbST	^{ab} 16.8 ± 2.3 mg/dL	13.5%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

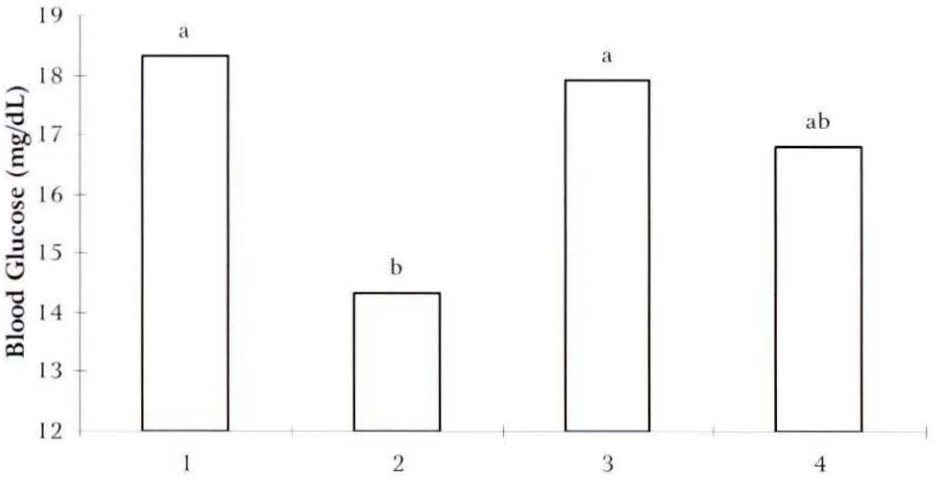

Figure 24. The insulin challenge maximum response

2.2.5 Time to reach the maximum glucose response

The time to minimum whole-blood glucose concentration was recorded individually for each insulin challenge and affected by treatments ($P < 0.0112$). These differences are presented in Table 25 and Figure 25 although it should be kept in mind that the difference between periods 1 and 4 reached statistical significance ($P < 0.05$), due to rounding of the probability, which was strictly speaking only a tendency toward significance ($P < 0.0528$). The significant decrease in the timing of the rbST response also lead to the fact that the hyperglycaemic clamp was delayed during this period as the whole-blood glucose concentration had not yet stabilized and not returned to baseline concentration by t210. There was also a tendency ($P < 0.0584$) for the difference between the rbST and 80% ME periods.

Table 25. The time to reach maximum glucose response

Period	Description	Mean ± SD	CV
1	Control	[a] 42.0 ± 6.7 minutes	16.1%
2	rbST	[b] 48.5 ± 7.5 minutes	15.4%
3	80% ME	[ab] 42.5 ± 6.8 minutes	15.9%
4	80% ME + rbST	[b] 46.0 ± 5.2 minutes	11.2%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

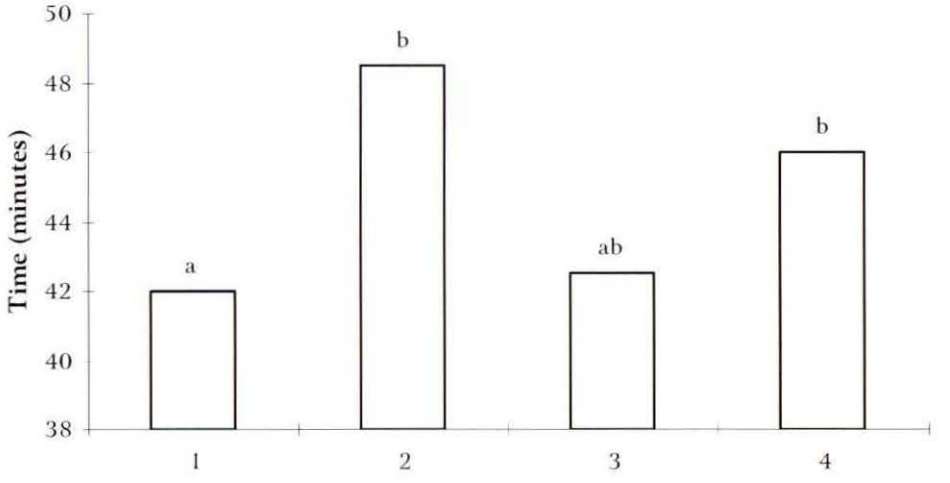

Figure 25. The time to reach maximum response

2.3 Data collected during the hyperglycaemic clamp

2.3.1 The control period

The glucose concentrations in whole-blood periodically determined from t–30 before glucose injection, up to up to t120 minutes after initiation of glucose infusion are represented as a weighted average (n=10) at each time point (Table 26 and Figure 26).

Table 26. Glucose clamp whole-blood glucose concentration (control)

Time	Mean ± SD	CV
t –30	46.9 ± 3.6 mg/dL	7.7%
t –20	47.1 ± 3.9 mg/dL	8.2%
t –10	47.8 ± 4.4 mg/dL	9.2%
t 0	48.0 ± 4.6 mg/dL	9.6%
t 5	60.9 ± 5.3 mg/dL	8.8%
t 10	70.1 ± 5.4 mg/dL	7.7%
t 15	77.7 ± 7.2 mg/dL	9.3%
t 20	84.7 ± 7.7 mg/dL	9.1%
t 25	88.6 ± 7.3 mg/dL	8.2%
t 30	92.9 ± 7.8 mg/dL	8.4%
t 40	95.9 ± 6.5 mg/dL	6.8%
t 50	96.6 ± 6.7 mg/dL	7.0%
t 60	97.7 ± 6.9 mg/dL	7.0%
t 70	97.4 ± 6.3 mg/dL	6.5%
t 80	97.2 ± 5.6 mg/dL	5.8%
t 90	97.2 ± 4.7 mg/dL	4.8%
t 100	96.1 ± 4.9 mg/dL	5.1%
t 110	95.1 ± 4.9 mg/dL	5.1%
t 120	94.5 ± 4.3 mg/dL	4.5%

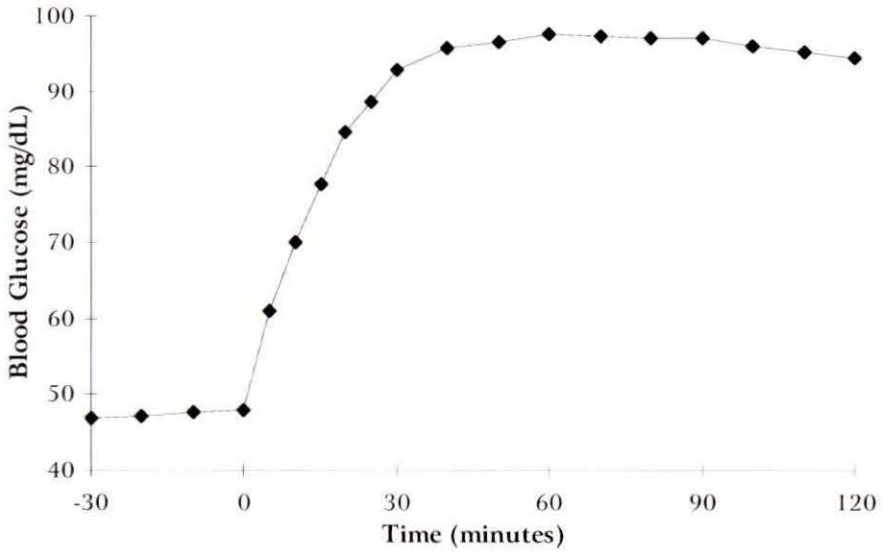

Figure 26. Glucose clamp whole-blood concentration (control)

2.3.2 The rbST period

The mean whole-blood glucose concentrations for each time point of the hyperglycaemic clamp, performed in the rbST period are presented in Table 27 and Figure 27.

Table 27. Glucose clamp whole-blood glucose concentration (rbST)

Time	Mean ± SD	CV
t –30	41.1 ± 4.3 mg/dL	10.5%
t –20	41.4 ± 4.5 mg/dL	10.8%
t –10	41.6 ± 4.8 mg/dL	11.5%
t 0	42.0 ± 4.9 mg/dL	11.7%
t 5	54.3 ± 4.6 mg/dL	8.5%
t 10	63.0 ± 5.8 mg/dL	9.1%
t 15	70.1 ± 6.7 mg/dL	9.6%
t 20	77.3 ± 6.6 mg/dL	8.6%
t 25	83.2 ± 6.2 mg/dL	7.5%
t 30	87.9 ± 7.1 mg/dL	8.1%
t 40	89.1 ± 7.8 mg/dL	8.8%
t 50	91.5 ± 7.3 mg/dL	8.0%
t 60	93.0 ± 7.7 mg/dL	8.3%
t 70	92.9 ± 6.4 mg/dL	6.9%
t 80	93.8 ± 7.0 mg/dL	7.4%
t 90	94.0 ± 7.3 mg/dL	7.7%
t 100	93.8 ± 6.1 mg/dL	6.5%
t 110	93.2 ± 6.7 mg/dL	7.2%
t 120	91.8 ± 5.5 mg/dL	6.0%

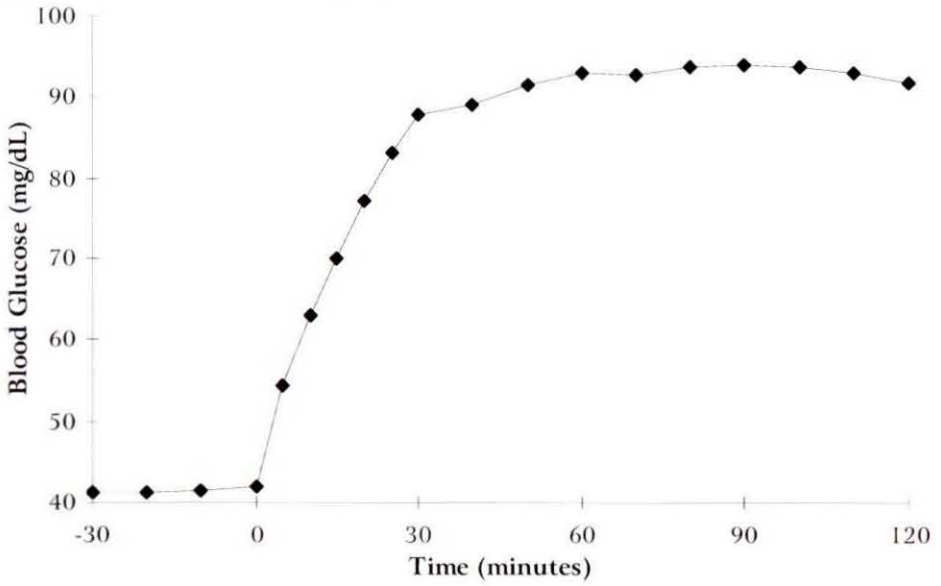

Figure 27. Glucose clamp whole-blood concentration (rbST)

2.3.3 The 80% ME period

The collection of whole-blood glucose concentration data followed 1 week after initiation of energy restriction to 80% of the estimated ME requirement. Data is presented in Table 28 and Figure 28.

Table 28. Glucose clamp whole-blood glucose concentration (80% ME)

Time	Mean ± SD	CV
t –30	45.0 ± 4.2 mg/dL	9.4%
t –20	45.6 ± 3.7 mg/dL	8.2%
t –10	45.3 ± 4.1 mg/dL	9.0%
t 0	46.0 ± 4.9 mg/dL	10.6%
t 5	59.1 ± 4.4 mg/dL	7.4%
t 10	67.2 ± 4.3 mg/dL	6.5%
t 15	75.5 ± 4.4 mg/dL	5.9%
t 20	81.5 ± 5.3 mg/dL	6.5%
t 25	89.1 ± 6.3 mg/dL	7.1%
t 30	93.3 ± 4.7 mg/dL	5.0%
t 40	94.0 ± 5.4 mg/dL	5.7%
t 50	95.2 ± 5.6 mg/dL	5.9%
t 60	96.2 ± 5.0 mg/dL	5.2%
t 70	95.6 ± 5.0 mg/dL	5.2%
t 80	95.3 ± 4.8 mg/dL	5.0%
t 90	96.6 ± 6.2 mg/dL	6.4%
t 100	95.6 ± 5.5 mg/dL	5.7%
t 110	94.4 ± 6.3 mg/dL	6.7%
t 120	92.9 ± 6.1 mg/dL	6.6%

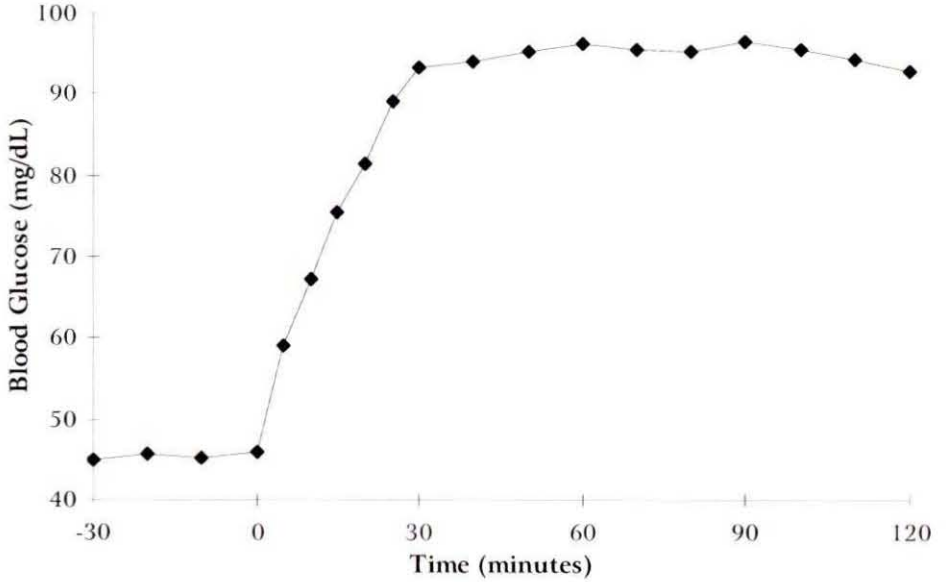

Figure 28. Glucose clamp whole-blood concentration (80% ME)

2.3.4 The 80% ME + rbST period

The mean whole-blood glucose concentrations determined from t–30 to t120 of hyperglycaemia in the 80% ME + rbST period are presented in Table 29 and Figure 29.

Table 29. Glucose clamp whole-blood glucose concentration (80% ME + rbST)

Time	Mean ± SD	CV
t –30	43.9 ± 2.9 mg/dL	6.7%
t –20	44.2 ± 2.5 mg/dL	5.6%
t –10	44.7 ± 3.3 mg/dL	7.3%
t 0	45.3 ± 3.2 mg/dL	7.0%
t 5	58.0 ± 3.7 mg/dL	6.4%
t 10	68.7 ± 3.3 mg/dL	4.8%
t 15	76.1 ± 4.2 mg/dL	5.5%
t 20	83.5 ± 4.7 mg/dL	5.6%
t 25	90.0 ± 4.5 mg/dL	5.0%
t 30	92.7 ± 4.5 mg/dL	4.9%
t 40	94.5 ± 4.9 mg/dL	5.2%
t 50	96.2 ± 6.4 mg/dL	6.7%
t 60	97.1 ± 5.9 mg/dL	6.1%
t 70	97.6 ± 4.3 mg/dL	4.4%
t 80	97.8 ± 4.9 mg/dL	5.0%
t 90	97.9 ± 5.5 mg/dL	5.6%
t 100	98.0 ± 5.9 mg/dL	6.0%
t 110	97.0 ± 5.7 mg/dL	5.8%
t 120	95.7 ± 4.7 mg/dL	5.0%

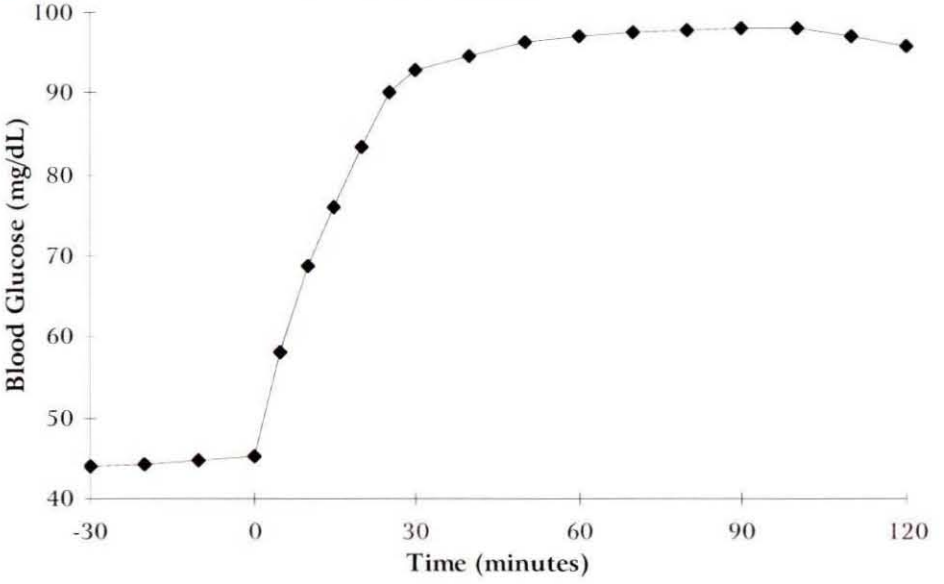

Figure 29. Glucose clamp whole-blood concentration (80% ME + rbST)

2.4 Effect of treatments in the hyperglycaemic clamp

2.4.1 The baseline glucose concentration

The individual whole-blood glucose concentrations determined at 10-minute intervals from t–30 to t0 were analyzed using repeated measures between periods (Table 30 and Figure 30). There was a small, but significant effect of time of sampling (t–30, t–20, t–10 and t0) on blood glucose concentrations ($P < 0.0011$), which is described in more detail in the Materials and Methods (paragraph 5.3.1). Treatments affected blood glucose concentrations of the baseline preceding the glucose clamp ($P < 0.0001$).

Table 30. The glucose clamp baseline whole-blood concentration

Period	Description	Mean ± SD	CV
1	Control	[a] 47.5 ± 4.0 mg/dL	8.4%
2	rbST	[b] 41.5 ± 4.4 mg/dL	10.7%
3	80% ME	[c] 45.5 ± 4.1 mg/dL	9.0%
4	80% ME + rbST	[d] 44.5 ± 2.9 mg/dL	6.6%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

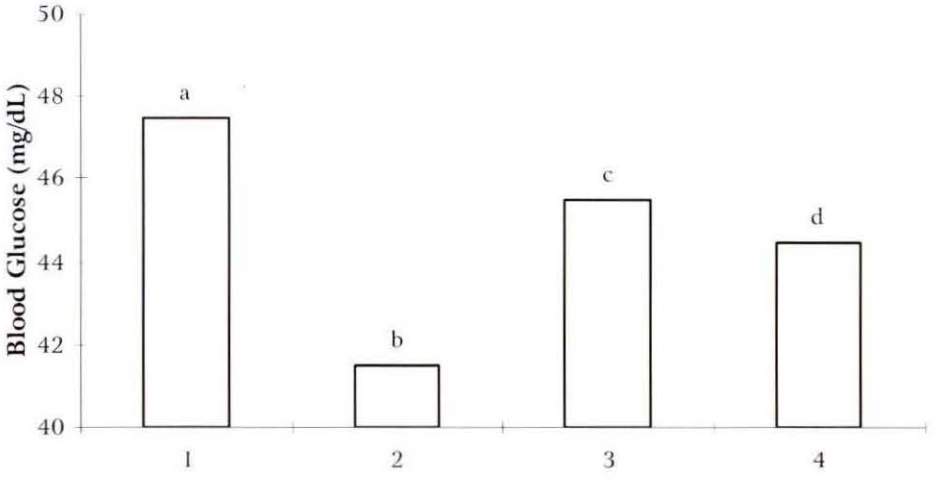

Figure 30. The glucose clamp baseline (whole-blood)

2.4.2 The total glucose infusion rate

The total amount of glucose infused during the entire clamp (120 minutes) was unaffected by period ($P < 0.7114$), as it included the rise toward clamped hyperglycaemia, where experimental protocols were similar for all cows (Table 31 and Figure 31).

Table 31. The glucose clamp total GIR

Period	Description	Mean ± SD	CV
1	Control	[a] 2.8 ± 0.4 mg / kg × min	15.1%
2	rbST	[a] 2.8 ± 0.4 mg / kg × min	15.6%
3	80% ME	[a] 2.9 ± 0.4 mg / kg × min	14.7%
4	80% ME + rbST	[a] 2.9 ± 0.4 mg / kg × min	13.9%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

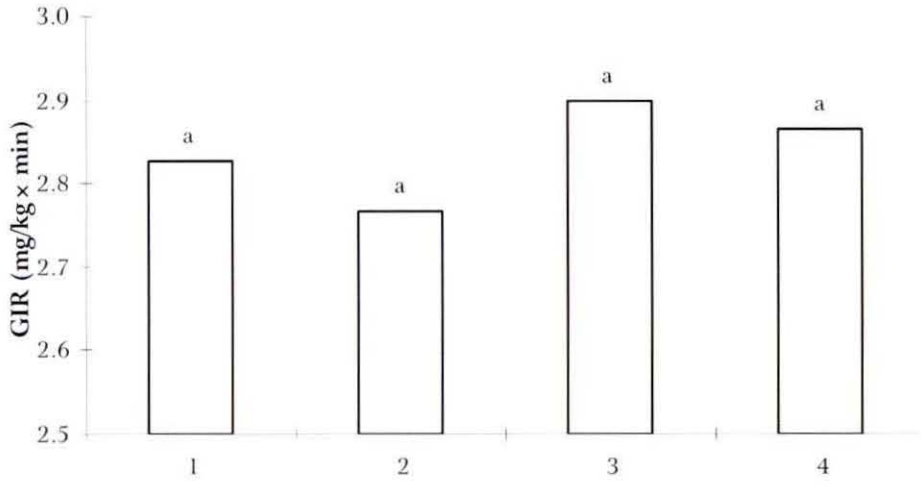

Figure 31. The glucose clamp total GIR

2.4.3 The steady-state glucose infusion rate

The amount (grams) of glucose infused during every 10-minute period of the final 40 minutes (t80 to t120) and the fasting body weight was used to calculate the SSGIR. The SSGIR was not affected by the individual time point measurements ($P < 0.1574$), but was affected by period ($P < 0.0001$) as presented in Table 32 and Figure 32. The difference between the control and 80% ME period tended to reach significance ($P < 0.0774$).

Table 32. The glucose clamp SSGIR

Period	Description	Mean ± SD	CV
1	Control	[a] 2.3 ± 0.5 mg / kg × min	19.9%
2	rbST	[b] 2.1 ± 0.4 mg / kg × min	19.6%
3	80% ME	[a] 2.3 ± 0.5 mg / kg × min	21.5%
4	80% ME + rbST	[c] 2.2 ± 0.5 mg / kg × min	23.3%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

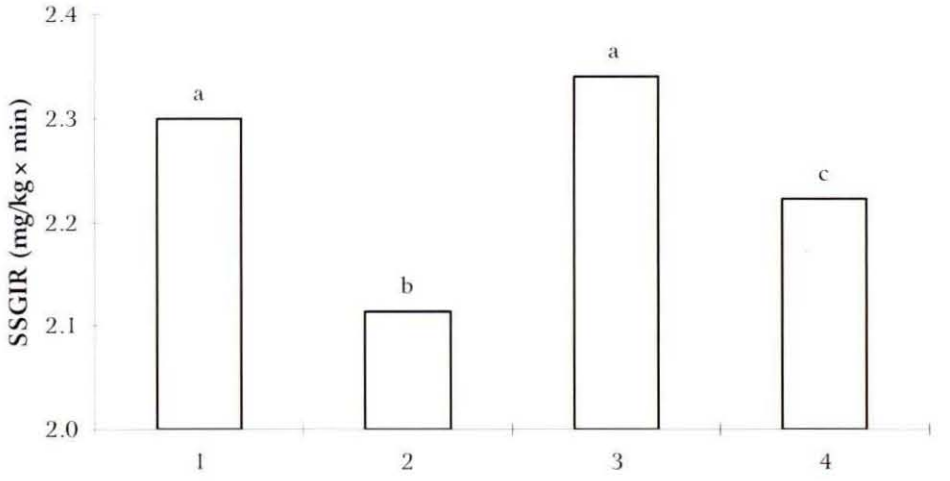

Figure 32. The glucose clamp SSGIR

3. Plasma data

3.1 Data collected during the basal period

3.1.1 Individual glucose concentrations

Plasma samples harvested after jugular venipuncture up to week 7 were analyzed for plasma glucose concentration. Plasma was collected fortnightly, on two consecutive days and data presented as the weighted average for all cows (n=10) for each sampling day (Table 33 and Figure 33).

Table 33. Individual basal plasma glucose concentrations

Day	Mean ± SD	CV
7	58.2 ± 8.3 mg/dL	14.3%
8	57.1 ± 6.0 mg/dL	10.6%
21	59.7 ± 5.0 mg/dL	8.4%
22	61.1 ± 4.9 mg/dL	8.0%
35	62.5 ± 4.7 mg/dL	7.4%
36	62.9 ± 2.4 mg/dL	3.9%
49	62.9 ± 2.9 mg/dL	4.5%
50	59.5 ± 2.6 mg/dL	4.3%

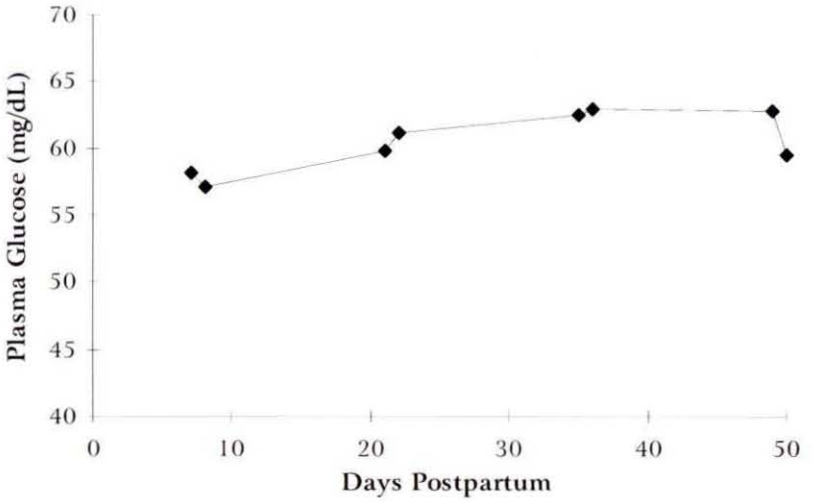

Figure 33. Individual basal plasma glucose concentration

3.1.2 Average glucose concentration

The weighted average of each week was calculated from the data presented in Table 33. The weekly average basal plasma glucose concentrations were calculated as a weighted average of the two consecutive samples and are presented in Table 34 and Figure 34.

Table 34. Mean basal plasma glucose concentrations

Week	Mean ± SD	CV
1	57.6 ± 6.4 mg/dL	11.1%
3	60.2 ± 4.8 mg/dL	8.0%
5	62.7 ± 3.0 mg/dL	4.7%
7	61.2 ± 2.5 mg/dL	4.0%

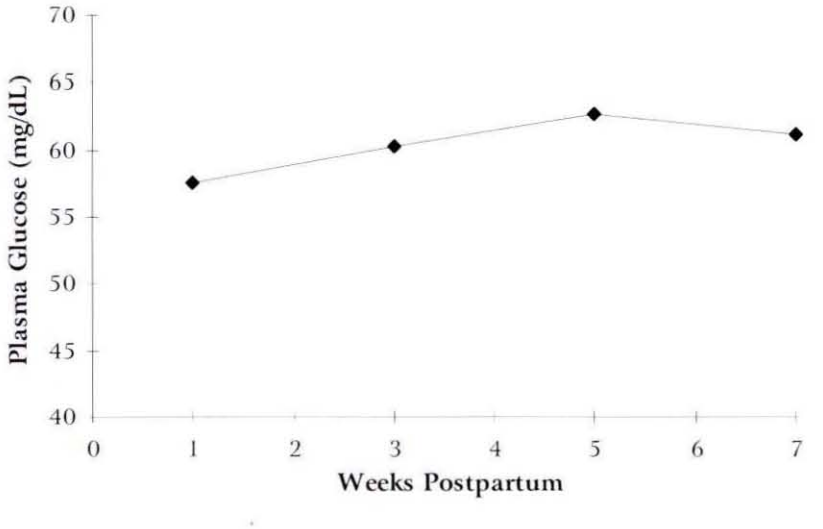

Figure 34. Mean basal plasma glucose concentration

3.2 Data collected during the insulin challenge

3.2.1 The control period

The glucose concentration in plasma was periodically determined from t–30 up to t150 minutes, relative to insulin injection. The data for the control period are presented in Table 35 and Figure 35. Values represent a weighted mean for all cows (n=10) for each plasma sample.

Table 35. Insulin challenge plasma glucose concentration (control)

Time	Mean ± SD	CV
t –30	66.4 ± 3.7 mg/dL	5.6%
t –20	67.3 ± 4.9 mg/dL	7.3%
t –10	67.7 ± 4.3 mg/dL	6.4%
t 0	66.9 ± 4.3 mg/dL	6.4%
t 30	45.3 ± 4.6 mg/dL	10.1%
t 60	50.1 ± 5.6 mg/dL	11.2%
t 90	57.0 ± 5.6 mg/dL	9.7%
t 120	59.9 ± 6.1 mg/dL	10.2%
t 150	62.9 ± 5.7 mg/dL	9.1%

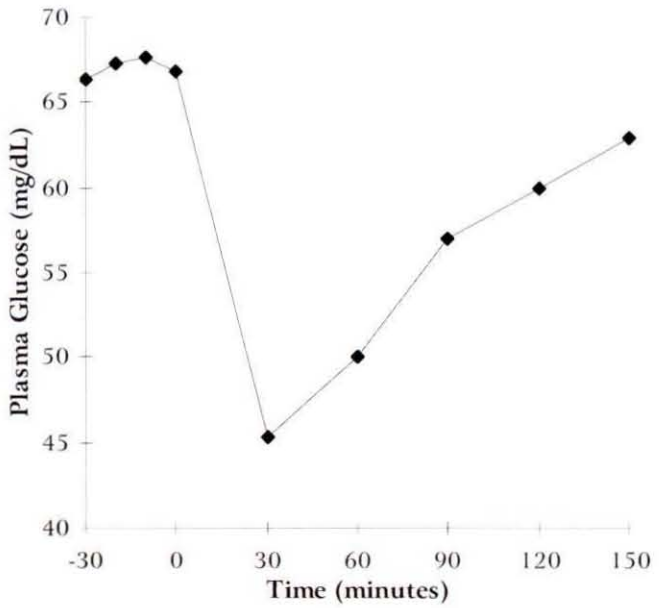

Figure 35. Insulin challenge plasma concentration (control)

3.2.2 The rbST period

The plasma glucose concentration data periodically collected after insulin injection, for the rbST treatment period are presented in Table 36 and Figure 36.

Table 36. Insulin challenge plasma glucose concentration (rbST)

Time	Mean ± SD	CV
t –30	60.0 ± 5.0 mg/dL	8.3%
t –20	60.3 ± 4.9 mg/dL	8.2%
t –10	60.3 ± 5.0 mg/dL	8.3%
t 0	60.8 ± 5.3 mg/dL	8.8%
t 30	43.8 ± 4.4 mg/dL	10.0%
t 60	44.8 ± 6.7 mg/dL	15.0%
t 90	49.6 ± 6.6 mg/dL	13.2%
t 120	53.8 ± 6.6 mg/dL	12.2%
t 150	55.9 ± 6.4 mg/dL	11.5%

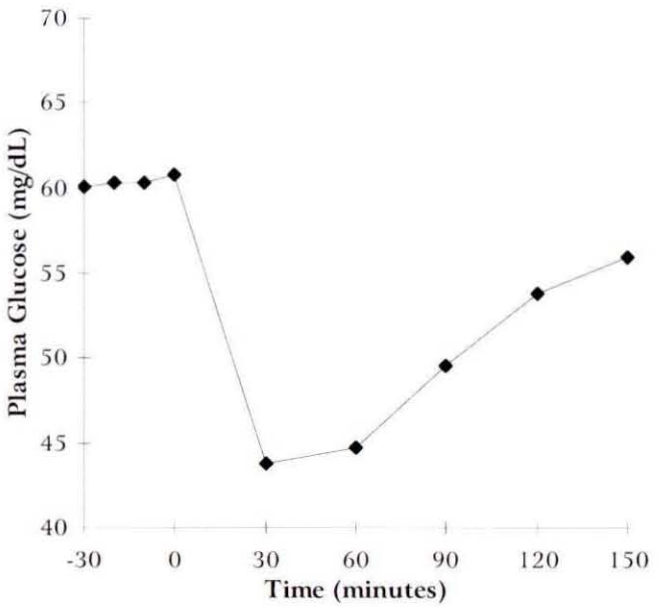

Figure 36. Insulin challenge plasma concentration (rbST)

3.2.3 The 80% ME period

Data of the 80% ME period was collected ± 7 days after energy restriction was commenced (Table 37 and Figure 37) and represents the average for all cows of plasma harvested in the insulin challenge.

Table 37. Insulin challenge plasma glucose concentration (80% ME)

Time	Mean ± SD	CV
t –30	63.9 ± 4.8 mg/dL	7.5%
t –20	64.2 ± 5.4 mg/dL	8.3%
t –10	63.9 ± 5.2 mg/dL	8.1%
t 0	63.1 ± 5.5 mg/dL	8.8%
t 30	42.5 ± 5.2 mg/dL	12.4%
t 60	46.1 ± 6.0 mg/dL	13.0%
t 90	54.5 ± 5.9 mg/dL	10.8%
t 120	57.5 ± 5.6 mg/dL	9.7%
t 150	58.6 ± 5.0 mg/dL	8.5%

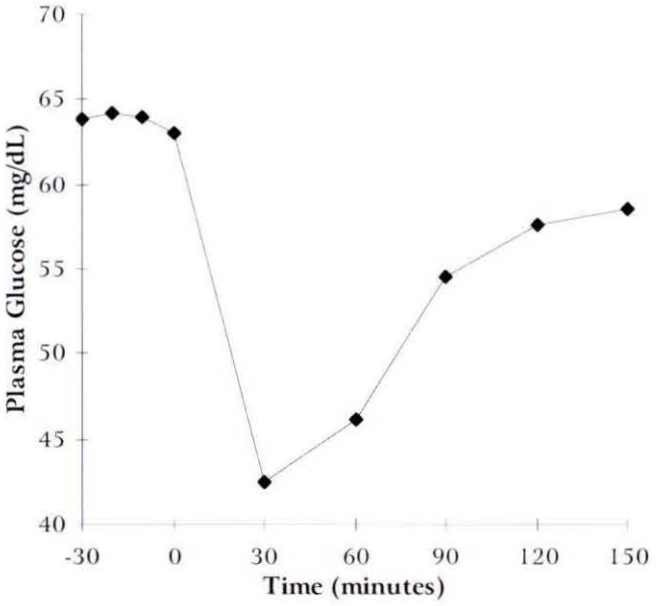

Figure 37. Insulin challenge plasma concentration (80% ME)

3.2.4 The 80% ME + rbST period

The glucose concentration in plasma was determined approximately 1 week after recombinant bST administration, during energy restriction (Table 38 and Figure 38).

Table 38. Insulin challenge plasma glucose concentration (80% ME + rbST)

Time	Mea	± SD	CV
t –30	63.7	± 4.1 mg/dL	6.4%
t –20	63.7	± 3.1 mg/dL	4.9%
t –10	65.1	± 4.1 mg/dL	6.4%
t 0	64.6	± 4.5 mg/dL	7.0%
t 30	45.7	± 3.4 mg/dL	7.4%
t 60	46.7	± 6.1 mg/dL	13.1%
t 90	53.8	± 5.9 mg/dL	10.9%
t 120	57.5	± 4.4 mg/dL	7.7%
t 150	59.2	± 3.2 mg/dL	5.5%

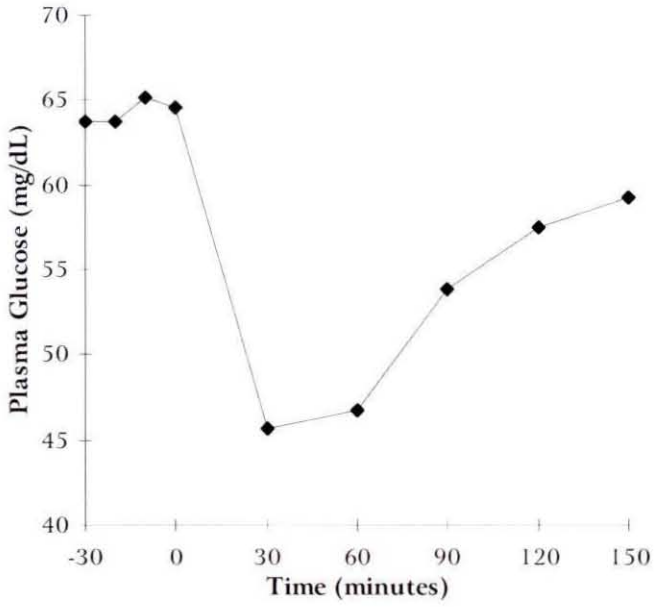

Figure 38. Insulin challenge plasma concentration (80% ME + rbST)

3.3 Effect of treatments in the insulin challenge

3.3.1 The baseline glucose concentration

The individual glucose concentrations determined at 10-minute intervals from t–30 to t0 were analyzed using repeated measures between periods. There was no significant effect of time of sampling ($P < 0.1409$) on glucose concentration, while period significantly affected the baseline plasma glucose concentrations ($P < 0.0001$). The small difference between the 80% ME and the 80% ME + rbST period (Table 39 and Figure 39) tended to reach significance ($P < 0.0755$).

Table 39. The insulin challenge baseline plasma concentration

Period	Description	Mean ± SD	CV
1	Control	[a] 67.1 ± 4.2 mg/dL	6.3%
2	rbST	[b] 60.4 ± 4.9 mg/dL	8.1%
3	80% ME	[c] 63.8 ± 5.0 mg/dL	7.9%
4	80% ME + rbST	[c] 64.3 ± 3.9 mg/dL	6.1%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

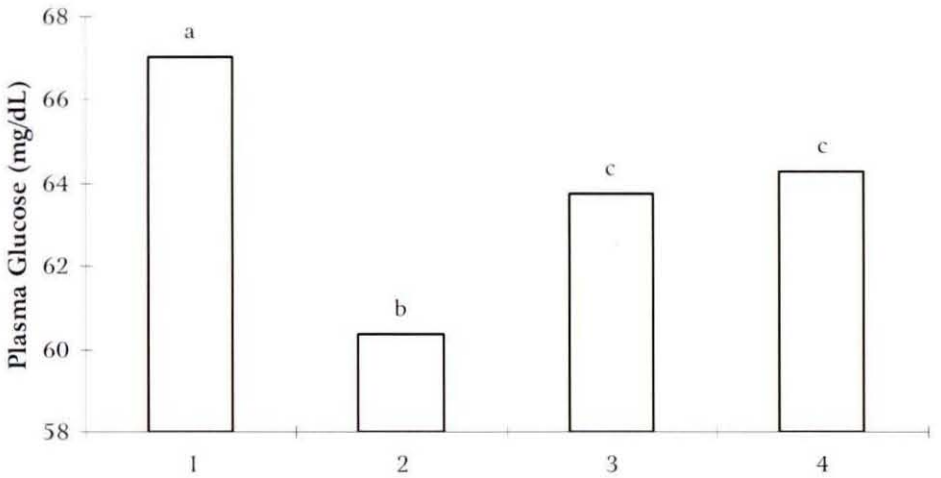

Figure 39. The insulin challenge baseline (plasma)

3.3.2 The baseline glucose AUC

The average baseline AUC calculated by summation of the areas of the trapezoids under the insulin challenge baseline (t–30 through t0) was affected by treatment ($P < 0.0004$) and results summarized in Table 40 and Figure 40.

Table 40. The insulin challenge baseline plasma AUC

Period	Description	Mean ± SD	CV
1	Control	[a] 2016.2 ± 128.8 mg × min /dL	6.4%
2	rbST	[b] 1810.5 ± 148.4 mg × min /dL	8.2%
3	80% ME	[c] 1915.7 ± 153.0 mg × min /dL	8.0%
4	80% ME + rbST	[ac] 1929.9 ± 111.9 mg × min /dL	5.8%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

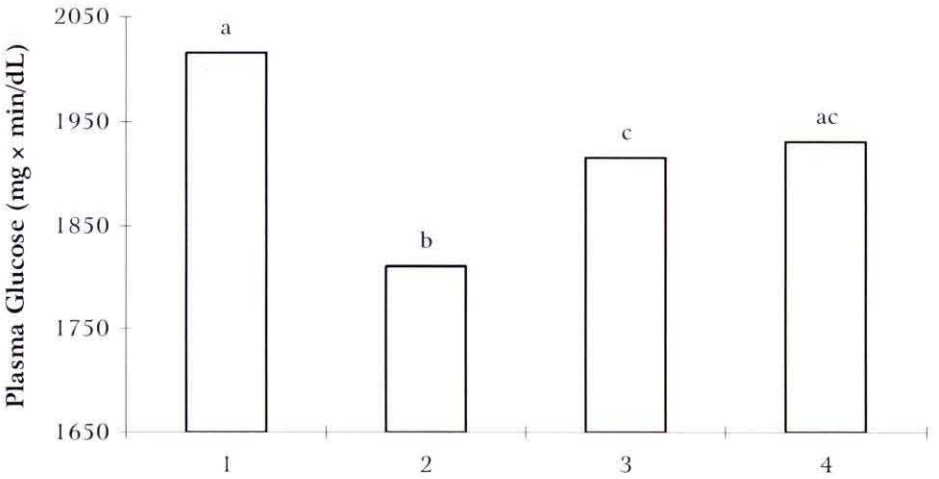

Figure 40. The insulin challenge baseline AUC (plasma)

3.3.3 The glucose AUC response to insulin injection (t0 - t30)

The AUC of the plasma glucose response phase was calculated by summation of the areas of the trapezoids between t0 to t30. These values were corrected for the baseline AUC to yield the corrected AUC above the curve. The differences between periods ($P < 0.0034$) are summarized in Table 41 and Figure 41. The difference between period 1 and period 4 reached statistical significance ($P < 0.05$), but it must be noted that this was due to rounding ($P < 0.0514$), while the difference between period 3 and period 4 tended to reach significance ($P < 0.0734$).

Table 41. The insulin challenge plasma response AUC

Period	Description	Mean ± SD	CV
1	Control	^{a} −333.8 ± 62.8 mg × min /dL	18.8%
2	rbST	^{b} −241.3 ± 60.3 mg × min /dL	25.0%
3	80% ME	^{ac} −333.1 ± 84.5 mg × min /dL	25.4%
4	80% ME + rbST	^{bc} −275.2 ± 37.9 mg × min /dL	13.8%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

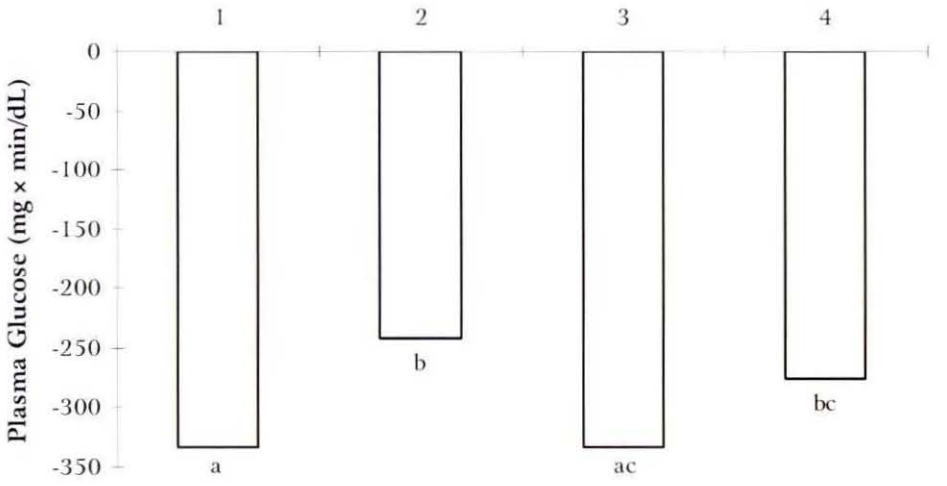

Figure 41. The insulin challenge response AUC (plasma)

3.4 Data collected during the hyperglycaemic clamp

3.4.1 The control period

The glucose concentration in plasma was periodically determined from t–30 to t120 minutes after initiation of glucose infusion, in the control period (Table 42 and Figure 42). Values are presented as the weighted average for all cows of each plasma sample ± SD and CV.

Table 42. Glucose clamp plasma glucose concentration (control)

Time	Mean ± SD	CV
t –30	64.6 ± 6.1 mg/dL	9.4%
t –20	64.5 ± 6.1 mg/dL	9.4%
t –10	65.4 ± 7.0 mg/dL	10.7%
t 0	65.7 ± 6.7 mg/dL	10.2%
t 20	115.4 ± 11.4 mg/dL	9.9%
t 40	130.3 ± 9.1 mg/dL	7.0%
t 60	132.7 ± 10.4 mg/dL	7.9%
t 80	133.8 ± 8.0 mg/dL	6.0%
t 90	133.9 ± 5.4 mg/dL	4.0%
t 100	132.0 ± 5.9 mg/dL	4.5%
t 110	130.3 ± 5.9 mg/dL	4.5%
t 120	129.5 ± 5.6 mg/dL	4.3%

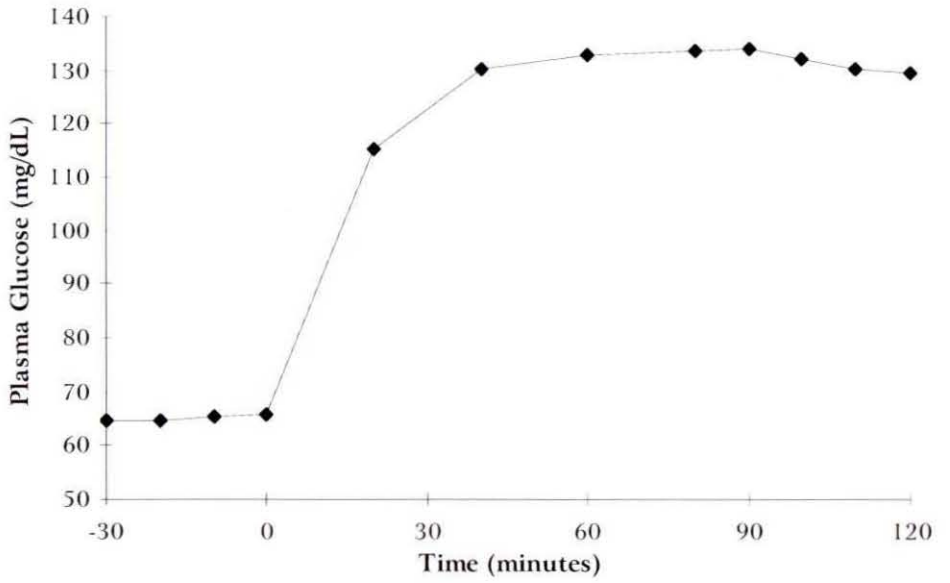

Figure 42. Glucose clamp plasma concentration (control)

3.4.2 The rbST period

Plasma glucose data collected for the rbST period is presented as the weighted average for each time point in Table 43 and Figure 43.

Table 43. Glucose clamp plasma glucose concentration (rbST)

Time	Mean ± SD	CV
t –30	55.4 ± 5.4 mg/dL	9.8%
t –20	55.8 ± 7.0 mg/dL	12.6%
t –10	55.3 ± 6.0 mg/dL	10.8%
t 0	54.9 ± 6.8 mg/dL	12.5%
t 20	105.1 ± 9.9 mg/dL	9.4%
t 40	121.1 ± 9.0 mg/dL	7.4%
t 60	125.3 ± 9.2 mg/dL	7.3%
t 80	125.3 ± 8.9 mg/dL	7.1%
t 90	126.6 ± 9.2 mg/dL	7.3%
t 100	127.2 ± 6.9 mg/dL	5.4%
t 110	128.0 ± 7.5 mg/dL	5.8%
t 120	125.4 ± 6.0 mg/dL	4.8%

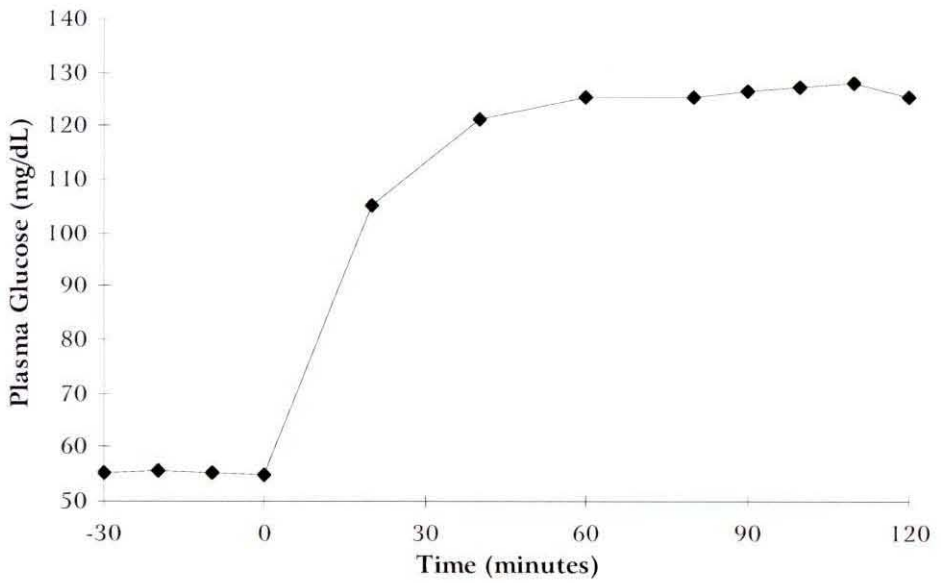

Figure 43. Glucose clamp plasma concentration (rbST)

3.4.3 The 80% ME period

The plasma glucose concentration of periodically collected samples of the 80% ME period is presented in Table 44 and Figure 44.

Table 44. Glucose clamp plasma glucose concentration (80% ME)

Time	Mean ± SD	CV
t –30	61.8 ± 6.4 mg/dL	10.3%
t –20	62.1 ± 4.6 mg/dL	7.4%
t –10	61.4 ± 5.5 mg/dL	8.9%
t 0	62.4 ± 6.5 mg/dL	10.4%
t 20	110.8 ± 6.4 mg/dL	5.7%
t 40	128.3 ± 6.5 mg/dL	5.1%
t 60	130.7 ± 6.1 mg/dL	4.7%
t 80	129.7 ± 7.0 mg/dL	5.4%
t 90	132.0 ± 8.0 mg/dL	6.0%
t 100	129.5 ± 7.8 mg/dL	6.1%
t 110	127.4 ± 11.0 mg/dL	8.6%
t 120	126.2 ± 10.7 mg/dL	8.4%

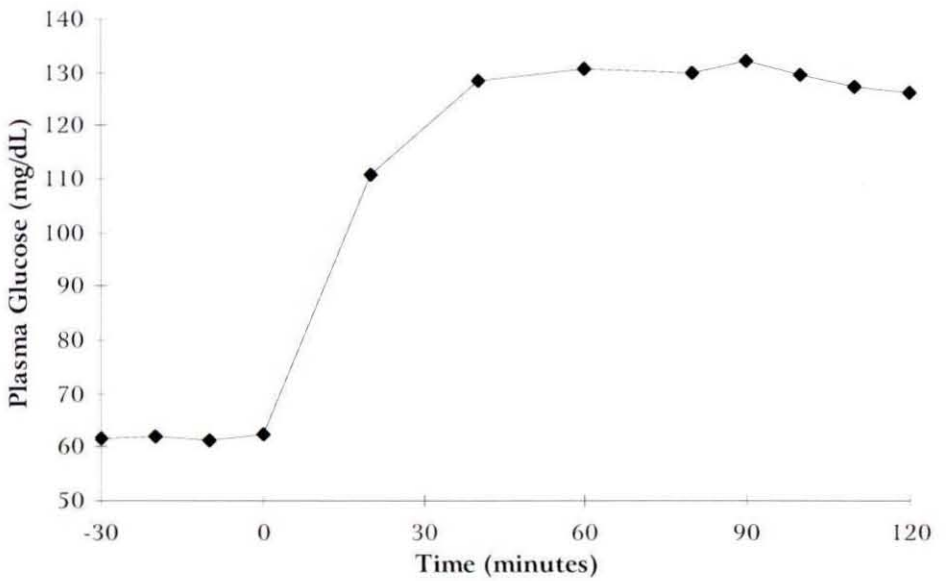

Figure 44. Glucose clamp plasma concentration (80% ME)

3.4.4 The 80% ME + rbST period

Approximately 1 week following recombinant bST injection during the energy restriction phase, plasma glucose samples were collected during 2 hours of hyperglycaemia and the mean plasma glucose concentration results are presented in Table 45 and Figure 45.

Table 45. Glucose clamp plasma glucose concentration (80% ME + rbST)

Time	Mean ± SD	CV
t –30	61.2 ± 3.3 mg/dL	5.4%
t –20	60.5 ± 3.5 mg/dL	5.7%
t –10	61.7 ± 4.7 mg/dL	7.6%
t 0	62.0 ± 4.6 mg/dL	7.4%
t 20	114.3 ± 6.2 mg/dL	5.4%
t 40	129.6 ± 7.3 mg/dL	5.6%
t 60	127.3 ± 7.7 mg/dL	6.1%
t 80	129.6 ± 6.8 mg/dL	5.2%
t 90	131.2 ± 8.4 mg/dL	6.4%
t 100	133.4 ± 6.7 mg/dL	5.0%
t 110	132.2 ± 6.9 mg/dL	5.2%
t 120	129.7 ± 7.2 mg/dL	5.5%

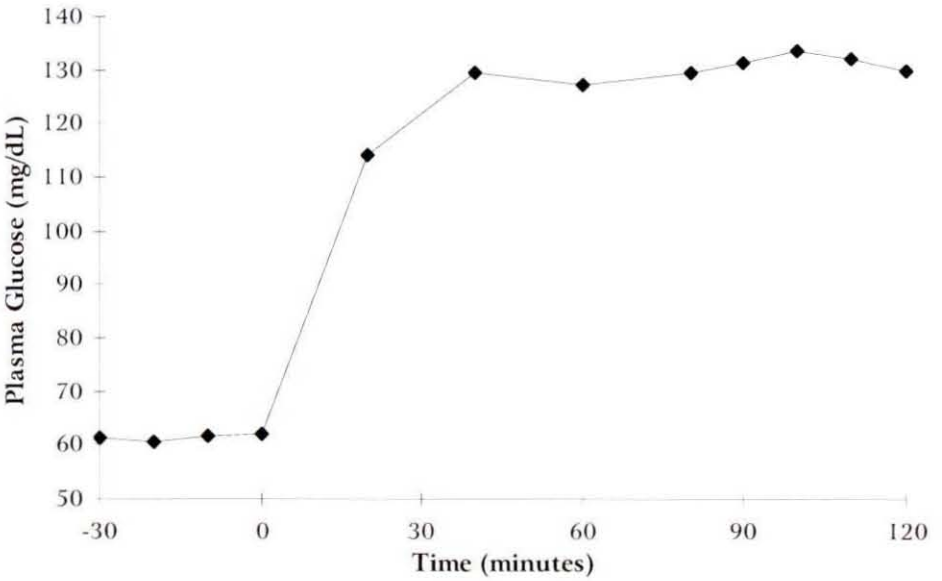

Figure 45. Glucose clamp plasma concentration (80% ME + rbST)

3.5 Effect of treatments in the hyperglycaemic clamp

3.5.1 The baseline glucose concentration

The individual plasma glucose concentrations determined at 10-minute intervals from t–30 up to t0 were analyzed using repeated measures between periods. These values were unaffected by the time of sampling ($P < 0.5195$), but significantly affected by treatment periods ($P < 0.0001$). This data is summarized in Table 46 and Figure 46.

Table 46. The glucose clamp baseline plasma concentration

Period	Description	Mean ± SD	CV
1	Control	[a] 65.0 ± 6.2 mg/dL	9.6%
2	rbST	[b] 55.4 ± 6.1 mg/dL	11.0%
3	80% ME	[c] 61.9 ± 5.6 mg/dL	9.0%
4	80% ME + rbST	[c] 61.4 ± 3.9 mg/dL	6.4%

Different superscripts (a,b,c,d) indicate statistically significant differences ($P < 0.05$) between periods.

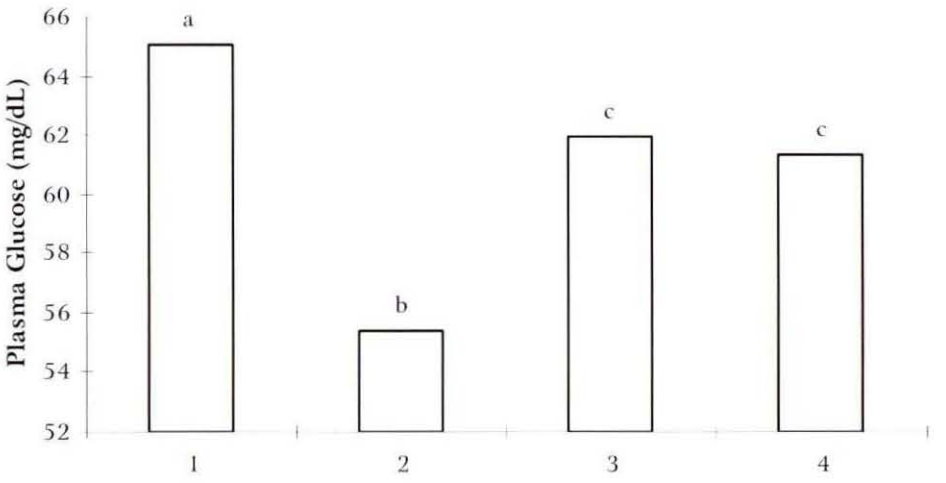

Figure 46. The glucose clamp baseline (plasma)

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%).

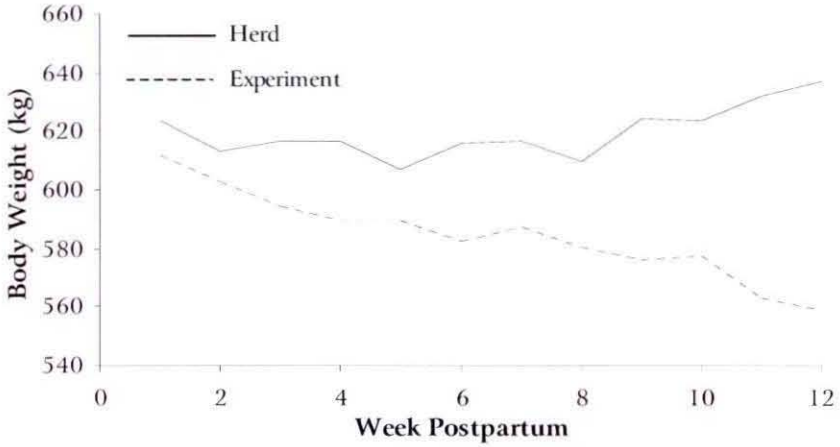

Figure 47. Body weight change in the herd

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.

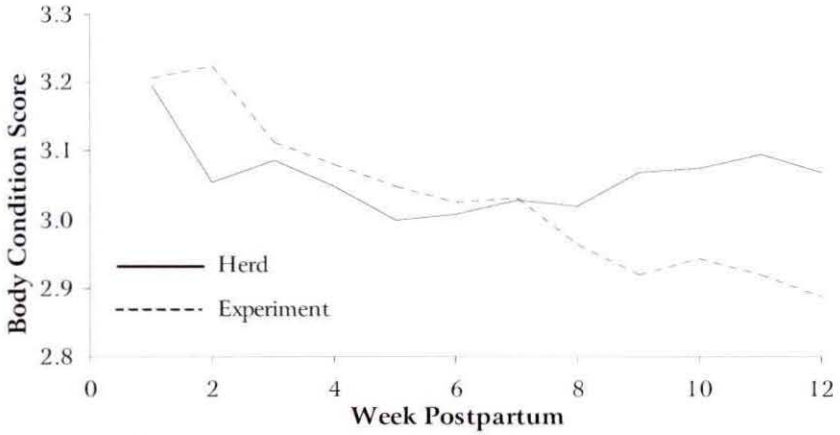

Figure 48. BCS change in the herd

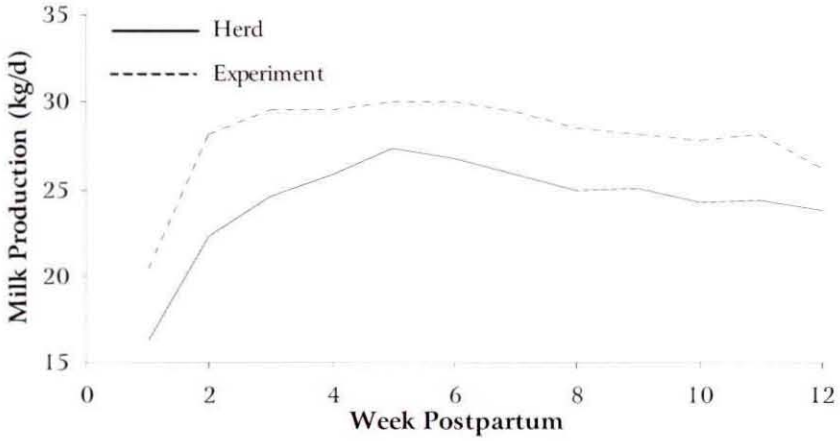

Figure 49. Milk production in the herd

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.

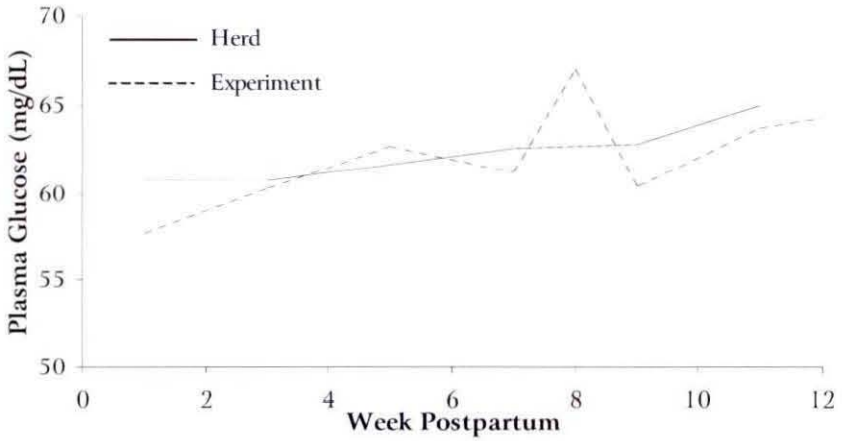

Figure 50. Plasma glucose concentration in the herd

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.

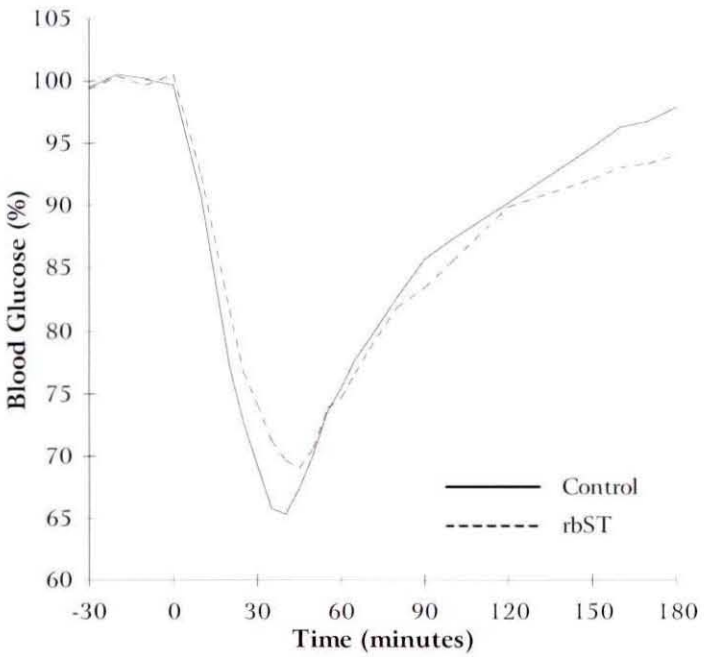

Figure 51. The effect of rbST on insulin challenge results

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).

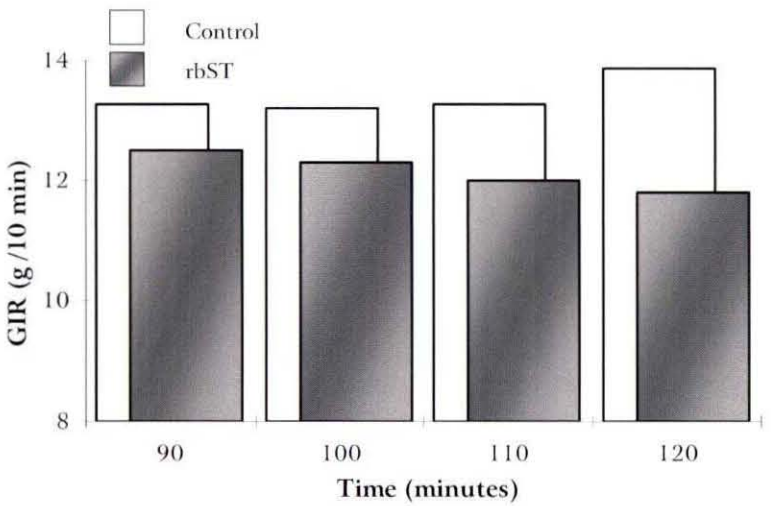

Figure 52. The effect of rbST on GIR in the steady-state period

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 ($P < 0.7976$).

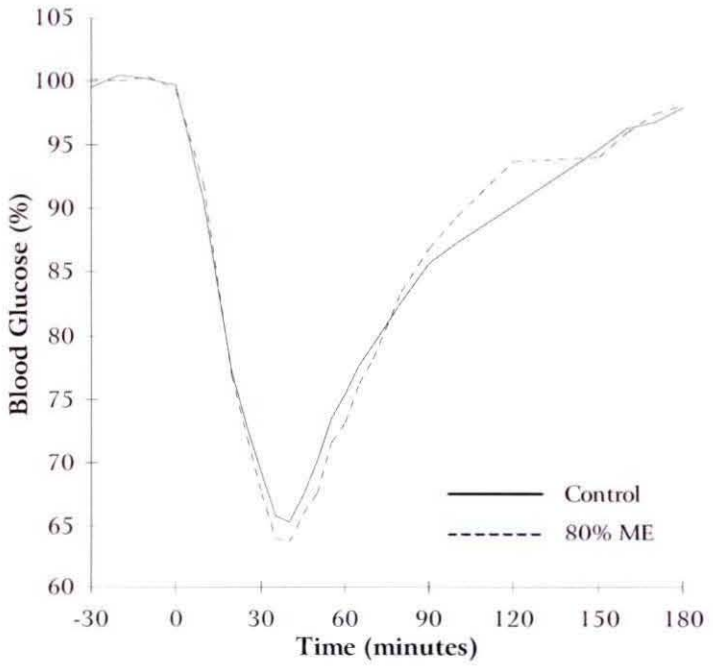

Figure 53. The effect of 80% ME on insulin challenge results

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 ($P<0.10$).

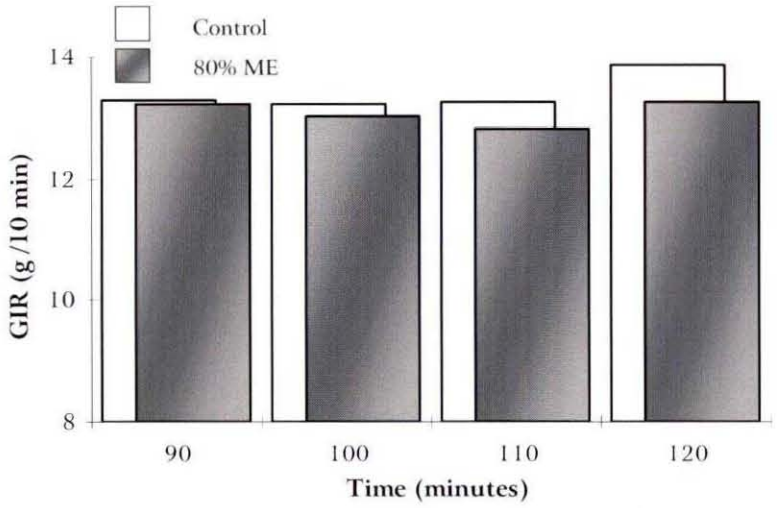

Figure 54. The effect of 80% ME on glucose clamp results

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.

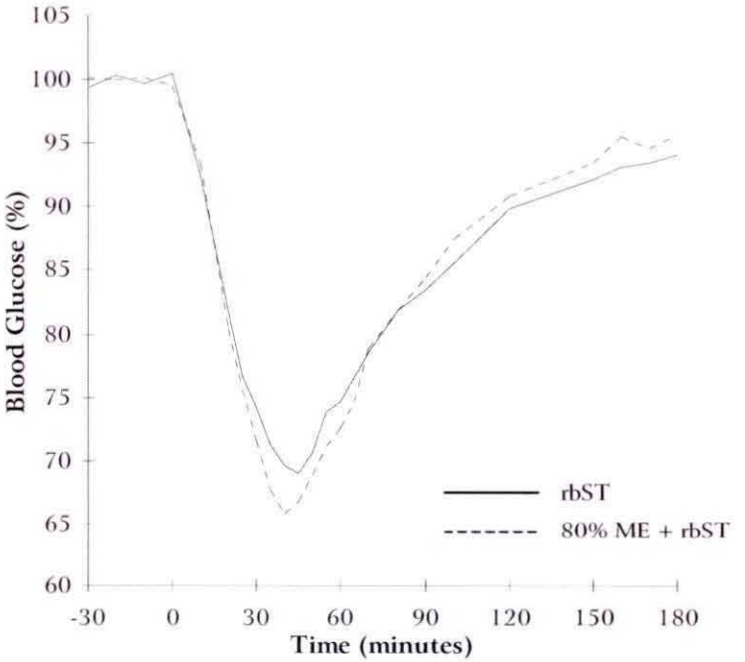

Figure 55. The effect of 80% ME on rbST insulin challenge results

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).

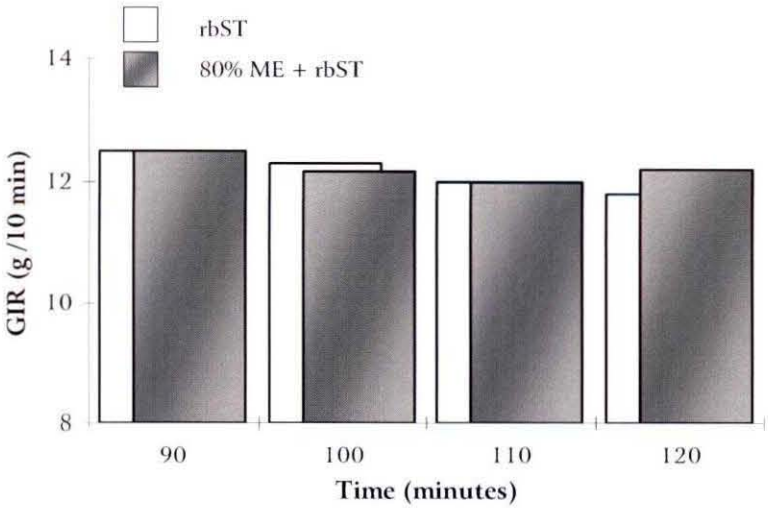

Figure 56. The effect of 80% ME on rbST glucose clamp results

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.

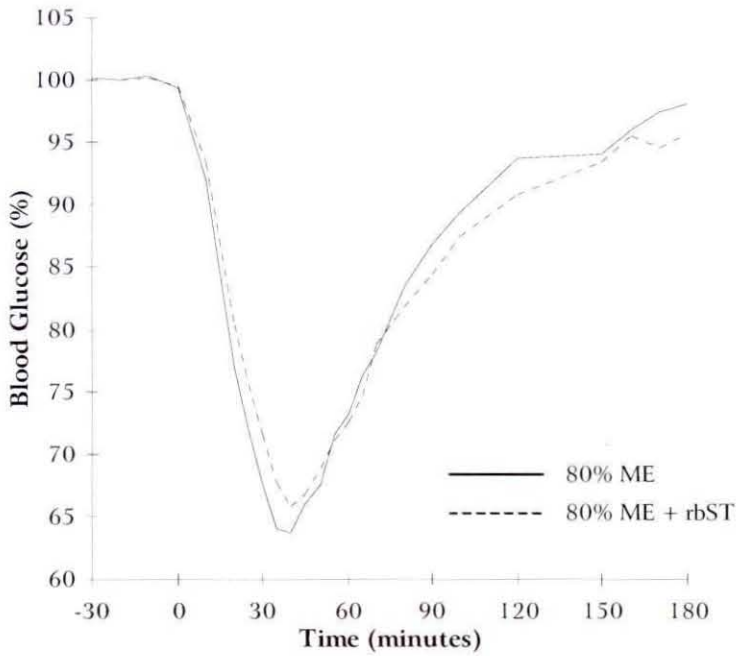

Figure 57. The effect of rbST on 80% ME insulin challenge results

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 × 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.

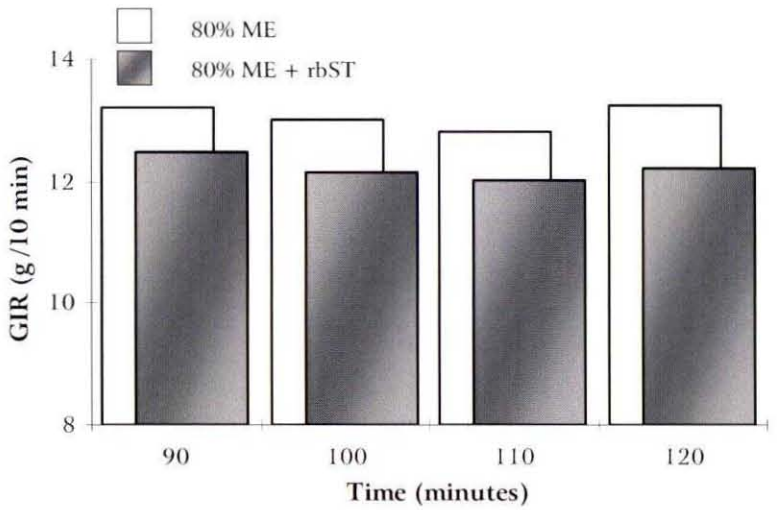

Figure 58. The effect of rbST on 80% ME glucose clamp results

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.

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