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).

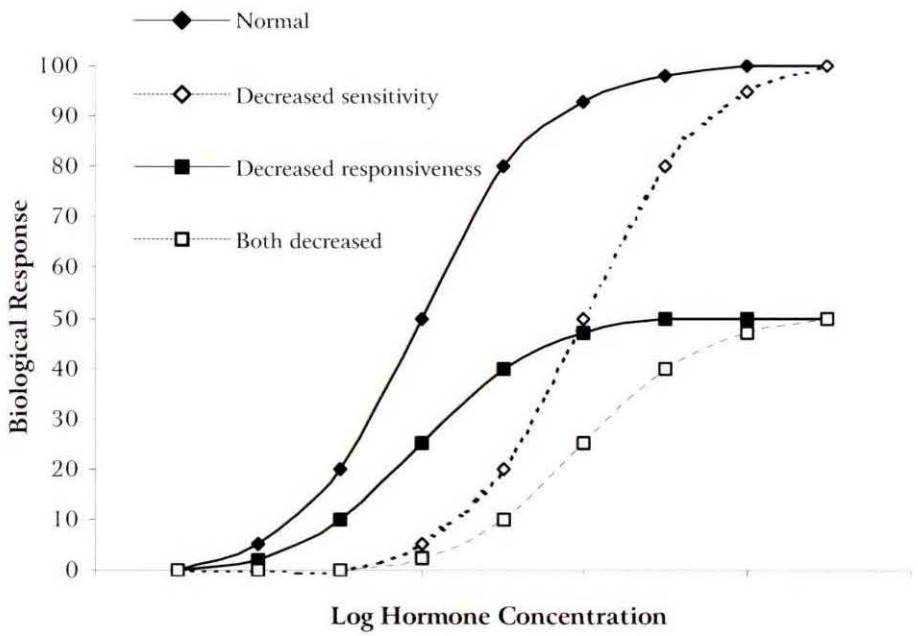

Figure 1. The components of hormone resistance (Kahn, 1978)

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).

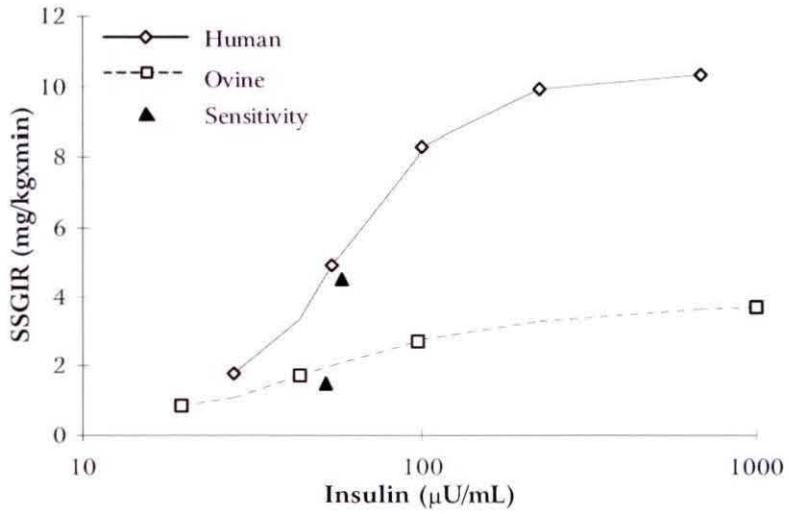

Figure 2. Dose-response curve for SSGIR in humans *vs.* sheep (adapted from Rizza *et al.*, 1981 and Weekes *et al.*, 1983)

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).

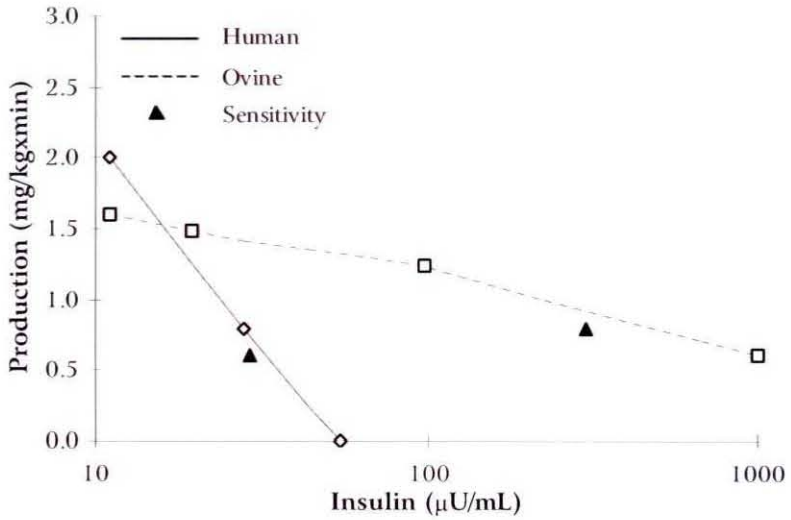

Figure 3. Dose-response curve for glucose production in humans *vs.* sheep (adapted from Rizza *et al.*, 1981 and Weekes *et al.*, 1983)

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).

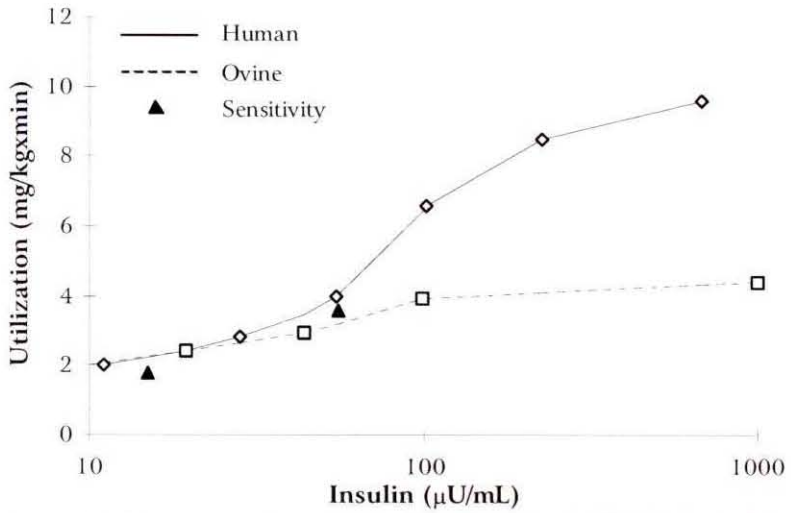

Figure 4. Dose-response curve for glucose utilization in humans *vs.* sheep (adapted from Rizza *et al.*, 1981 and Weekes *et al.*, 1983)

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.

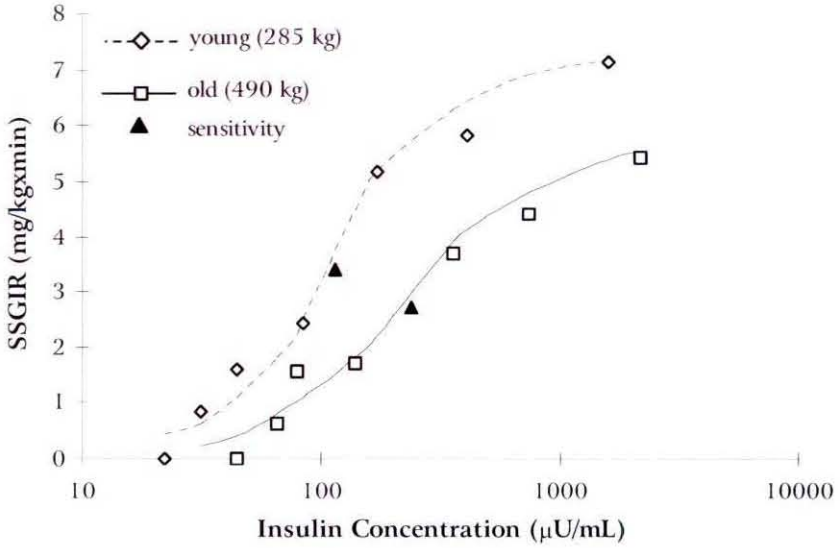

Figure 5. Dose-response curve for SSGIR in younger *vs.* older steers (Eisemann *et al.*, 1997)

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).

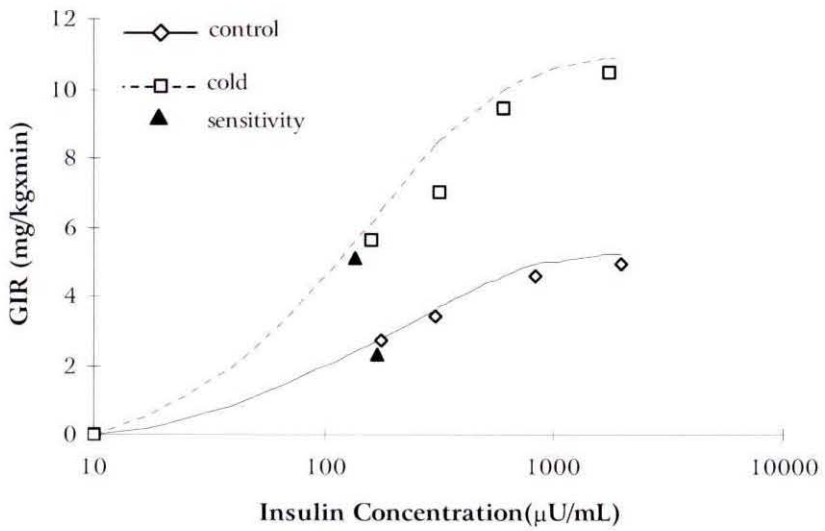

Figure 6. The effect of cold exposure on GIR (Achmadi *et al.*, 2001)

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).