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

by

Annelie Basson
BSc (Agric) Animal Science

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

June 2008

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

Annie Basson (94348449)

ABSTRACT

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

Keywords

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

Acknowledgements

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

SUMMARY

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

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

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

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

Summary of data collected during the experimental period

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

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

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

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SYMBOLS AND ABBREVIATIONS

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