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

by

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BSc (Agric) Animal Science

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University of Pretoria

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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.

[Signature]

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

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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 homeorhthetic 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 homeorhhetic 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

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Different superscripts (a,b,c,d) indicate statistically significant differences (P < 0.05) between periods
# TABLE OF CONTENTS

Abstract ......................................................................................................................... i

Keywords ...................................................................................................................... ii

Acknowledgements ..................................................................................................... ii

Summary .................................................................................................................... iii

Summary of data collected during the experimental period ..................................... v

Chapter 1. Introduction ................................................................................................. 1

Chapter 2. Literature review ......................................................................................... 3

1. Metabolic tests .......................................................................................................... 3
   1.1 Domestic ruminant vs. monogastric animals .................................................. 3
   1.2 Other notable effects ....................................................................................... 9
      1.2.1 Feeding and interaction with physiological state ................................... 9
      1.2.2 Body weight, adiposity and stage of the growth phase ......................... 11
      1.2.3 Temperature ......................................................................................... 12
      1.2.4 Acidosis and ketosis ............................................................................ 14
      1.2.5 Other .................................................................................................... 15

2. Somatotropin administration in lactation ............................................................. 15
   2.1 Somatotropin concentration ......................................................................... 16
   2.2 Production responses ...................................................................................... 17
      2.2.1 Milk production .................................................................................... 17
      2.2.2 Milk fat ................................................................................................ 19
      2.2.3 Milk lactose .......................................................................................... 21
      2.2.4 Milk protein .......................................................................................... 22
      2.2.5 Milk energy and the efficiency of milk production ............................... 23
      2.2.6 Milk minerals ....................................................................................... 25
   2.3 Metabolic responses ......................................................................................... 25
      2.3.1 Glucose, insulin and glucagon concentrations .................................... 25
      2.3.2 Glucose metabolism ............................................................................ 27
      2.3.3 Lipid metabolism .................................................................................. 30
      2.3.4 Protein metabolism .............................................................................. 33
   2.4 Effect on enzymes ............................................................................................ 35
      2.4.1 Gluconeogenesis .................................................................................. 36
      2.4.2 Lipogenesis ........................................................................................... 36
      2.4.3 Lipolysis and oxidation ........................................................................ 39
Chapter 3. Materials and methods

1. Animals ............................................................... 64
   1.1 Introduction .................................................... 64
   1.2 Cows included in the data analysis ....................... 65
   1.3 Cows excluded from the data analysis ................. 66

2. Periods and treatments ........................................ 68
   2.1 The basal period .............................................. 68
   2.2 The experimental period .................................. 68
      2.2.1 The control period .................................. 68
      2.2.2 The recombinant bovine somatotropin period (rbST) ........................................ 68
      2.2.3 The restriction to 80% of metabolizable energy requirement period (80% ME) .......... 68
      2.2.4 The 80% ME and rbST period (80% ME + rbST) ......................... 69

3. Data collection and processing ................................ 69
   3.1 Body weight, body condition score and milk production ........................................ 69
   3.2 Blood sampling .................................................. 69
   3.3 Catheterization ............................................... 70
   3.4 Insulin challenge ............................................. 71
3.5 Hyperglycaemic clamp ................................................................. 71
3.6 Recombinant bovine somatotropin injection ................................. 72
3.7 Calculations .................................................................................. 73
    3.7.1 Glucose infusion rate .......................................................... 73
    3.7.2 Glucose area under the curve ............................................... 73
    3.7.3 Maximum glucose response ................................................ 74
4. Sampling protocols ........................................................................ 75
    4.1 Basal blood collection ................................................................ 75
    4.2 Challenges, clamps and intake restriction ................................... 75
    4.3 The insulin challenge protocol .................................................. 76
    4.4 The hyperglycaemic clamp protocol ......................................... 77
5. Sample analyses ............................................................................ 78
    5.1 Basal samples ........................................................................... 78
        5.1.1 Plasma glucose concentration ......................................... 78
        5.1.2 Plasma insulin concentration .......................................... 79
        5.1.3 Plasma IGF-I concentration ............................................. 80
    5.2 The insulin challenge ................................................................ 81
        5.2.1 Whole-blood glucose concentration .................................. 81
        5.2.2 Plasma glucose concentration ......................................... 82
        5.2.3 Plasma insulin concentration .......................................... 83
        5.2.4 Plasma IGF-I concentration ............................................. 83
    5.3 The hyperglycaemic clamp ....................................................... 84
        5.3.1 Whole-blood glucose concentration .................................. 84
        5.3.2 Plasma glucose concentration ......................................... 85
        5.3.3 Plasma insulin concentration .......................................... 86
        5.3.4 Plasma IGF-I concentration ............................................. 86
6. Sample assay techniques ................................................................ 87
    6.1 Glucose concentration ............................................................. 87
    6.2 Plasma insulin concentration .................................................... 89
    6.3 Plasma IGF-I concentration ..................................................... 90

Chapter 4. Data and statistical analyses ........................................... 92

1. Production data ........................................................................... 92
    1.1 Body weight ............................................................................ 92
    1.2 Body condition score ............................................................. 93
    1.3 Milk production ..................................................................... 94
2. Whole-blood data ......................................................................... 96
    2.1 Data collected during the insulin challenge ............................... 96
        2.1.1 The control period ......................................................... 96
        2.1.2 The rbST period .......................................................... 97
        2.1.3 The 80% ME period ...................................................... 98
        2.1.4 The 80% ME + rbST period ......................................... 99
2. Recombinant bovine somatotropin .................................................. 130
  2.1 The insulin challenge ............................................................. 130
  2.2 The hyperglycaemic clamp ......................................................... 132
3. Energy restriction ........................................................................ 134
  3.1 The insulin challenge .............................................................. 134
  3.2 The hyperglycaemic clamp .......................................................... 136
4. Recombinant BST in the face of energy restriction .............................. 136
  4.1 The insulin challenge ............................................................. 136
  4.2 The hyperglycaemic clamp ......................................................... 138
5. Energy restriction coupled to rbST administration ............................. 140
  5.1 The insulin challenge ............................................................. 140
  5.2 The hyperglycaemic clamp .......................................................... 141

Chapter 6. Conclusion and critical evaluation .......................................... 142

References .................................................................................... 145
LIST OF TABLES

Table 1. Description of cows included in the data set.........................................................65
Table 2. Description of cows excluded from the data set.....................................................66
Table 3. Days of basal sample collection............................................................................75
Table 4. Days of treatment periods and intake restriction...................................................76
Table 5. Timing of samples in the insulin challenge.............................................................77
Table 6. Timing of samples in the hyperglycaemic clamp....................................................78
Table 7. Variation in consecutive basal period plasma glucose samples............................79
Table 8. Samples assayed in the basal period .....................................................................80
Table 9. Samples assayed in the control period (insulin challenge) ....................................82
Table 10. Samples assayed in rbST and/or 80% ME periods (insulin challenge) ...............83
Table 11. Variation in individual baseline samples (hyperglycaemic clamp) ......................84
Table 12. Samples assayed in the control period (glucose clamp) ......................................86
Table 13. Samples assayed in rbST and/or 80% ME periods (glucose clamp) ....................87
Table 14. Weekly mean body weight ..................................................................................92
Table 15. Weekly mean BCS .............................................................................................93
Table 16. Weekly mean milk production ............................................................................94
Table 17. Insulin challenge whole-blood glucose concentration (control) .........................96
Table 18. Insulin challenge whole-blood glucose concentration (rbST) .........................97
Table 19. Insulin challenge whole-blood glucose concentration (80% ME) ......................98
Table 20. Insulin challenge whole-blood glucose concentration (80% ME + rbST) ..........99
Table 21. The insulin challenge baseline whole-blood concentration................................100
Table 22. The insulin challenge baseline whole-blood AUC ............................................101
Table 23. The insulin challenge whole-blood response AUC ...........................................102
Table 24. The insulin challenge maximum response .......................................................103
Table 25. The time to reach maximum glucose response ................................................104
Table 26. Glucose clamp whole-blood glucose concentration (control) .........................105
Table 27. Glucose clamp whole-blood glucose concentration (rbST) .............................106
Table 28. Glucose clamp whole-blood glucose concentration (80% ME) .......................107
Table 29. Glucose clamp whole-blood glucose concentration (80% ME + rbST) ...........108
Table 30. The glucose clamp baseline whole-blood concentration ................................109
Table 31. The glucose clamp total GIR ..........................................................................110
Table 32. The glucose clamp SSGIR .............................................................................111
Table 33. Individual basal plasma glucose concentrations .............................................112
Table 34. Mean basal plasma glucose concentrations .....................................................113
Table 35. Insulin challenge plasma glucose concentration (control) ...............................114
Table 46. Glucose clamp baseline plasma concentration ...................... 125
Table 45. Glucose clamp plasma glucose concentration
Table 44. Glucose clamp plasma glucose concentration
Table 43. Glucose clamp plasma glucose concentration (control) ..................
Table 42. Glucose clamp plasma glucose concentration (rbST) ..................
Table 39. The insulin challenge baseline plasma concentration ..................
Table 38. Insulin challenge plasma glucose concentration
Table 37. Insulin challenge plasma glucose concentration
Table 36. Insulin challenge plasma glucose concentration (80% ME) ..................
Table 35. Insulin challenge plasma glucose concentration (80% ME + rbST) ........
Table 34. The insulin challenge baseline plasma AUC ............................ 119
Table 33. The insulin challenge plasma response AUC ........................... 120
Table 32. Glucose clamp plasma glucose concentration (control) ............... 121
Table 31. Glucose clamp plasma glucose concentration (rbST) ................... 122
Table 30. Glucose clamp plasma glucose concentration (80% ME) ............... 123
Table 29. Glucose clamp plasma glucose concentration (80% ME + rbST) ....... 124
Table 28. The glucose clamp baseline plasma concentration ................... 125

LIST OF FIGURES

Figure 1. The components of hormone resistance (Kahn, 1978) ..................... 4
Figure 2. Dose-response curve for SSGIR in humans vs. sheep (adapted from Rizza et al., 1981 and Weekes et al., 1983) ................ ........................................ 6
Figure 3. Dose-response curve for glucose production in humans vs. sheep (adapted from Rizza et al., 1981 and Weekes et al., 1983) ................ ........................................ 8
Figure 4. Dose-response curve for glucose utilization in humans vs. sheep (adapted from Rizza et al., 1981 and Weekes et al., 1983) ................ ........................................ 8
Figure 5. Dose-response curve for SSGIR in younger vs. older steers (Eisemann et al., 1997) ............ 12
Figure 6. The effect of cold exposure on GIR (Achmadi et al., 2001) ................ 13
Figure 7. Diagrammatic representation of experimental protocol .................. 64
Figure 8. Insulin challenge results of cows with suspected ketosis ................ 67
Figure 9. The area of a trapezium between two observations ..................... 74
Figure 10. Diagrammatic representation of glucose assay (YSI, 1997) ......... 88
Figure 11. Diagrammatic representation of the insulin ELISA (DRG, 2001) ...... 90
Figure 12. Diagrammatic representation of the IGF-I ELISA (IDS, 2001) .... 91
Figure 13. Weekly mean body weight .................................................. 92
Figure 14. Weekly mean body condition score ....................................... 93
Figure 15. Weekly mean milk production ............................................... 94
Figure 16. Mean milk production prior to metabolic tests ......................... 95
Figure 17. Insulin challenge whole-blood concentration (control) ............. 96
Figure 18. Insulin challenge whole-blood concentration (rbST) .................. 97
Figure 19. Insulin challenge whole-blood concentration (80% ME) ............ 98
Figure 20. Insulin challenge whole-blood concentration (80% ME + rbST) ....... 99
Figure 21. The insulin challenge baseline (whole-blood) ......................... 100
<table>
<thead>
<tr>
<th>Figure</th>
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<td>124</td>
</tr>
<tr>
<td>46</td>
<td>The glucose clamp baseline (plasma)</td>
<td>125</td>
</tr>
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<td>47</td>
<td>Body weight change in the herd</td>
<td>127</td>
</tr>
<tr>
<td>48</td>
<td>BCS change in the herd</td>
<td>127</td>
</tr>
<tr>
<td>49</td>
<td>Milk production in the herd</td>
<td>127</td>
</tr>
<tr>
<td>50</td>
<td>Plasma glucose concentration in the herd</td>
<td>129</td>
</tr>
<tr>
<td>51</td>
<td>The effect of rbST on insulin challenge results</td>
<td>131</td>
</tr>
<tr>
<td>52</td>
<td>The effect of rbST on GIR in the steady-state period</td>
<td>133</td>
</tr>
<tr>
<td>53</td>
<td>The effect of 80% ME on insulin challenge results</td>
<td>135</td>
</tr>
<tr>
<td>54</td>
<td>The effect of 80% ME on glucose clamp results</td>
<td>136</td>
</tr>
<tr>
<td>55</td>
<td>The effect of 80% ME on rbST insulin challenge results</td>
<td>138</td>
</tr>
<tr>
<td>56</td>
<td>The effect of 80% ME on rbST glucose clamp results</td>
<td>139</td>
</tr>
<tr>
<td>57</td>
<td>The effect of rbST on 80% ME insulin challenge results</td>
<td>140</td>
</tr>
<tr>
<td>58</td>
<td>The effect of rbST on 80% ME glucose clamp results</td>
<td>141</td>
</tr>
</tbody>
</table>
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
\( ^\circ C \) - degrees Celsius (centigrade)
\( A_{260} \) - absorbance at 260 nm
AUC - area under the (response) curve
BCS - body condition score
BP - binding proteins
bpST - 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\(_{6\,H_2O}\) - glucose monohydrate
GIR - glucose infusion rate
GLUT - facilitated diffusion hexose transporter
G\(_{protein}\) - guanosine triphosphate binding protein, inhibits (Gi) or stimulates (Gs) adenyl 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\(\times\)min - milligrams per kg body weight, per minute
min - minute
MJ - megajoule (where 1 joule is 0.2389 calories)
mol - moles \((6.02 \times 10^{23} \text{ per mol})\)
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 \((P < 0.05 \text{ considered statistically significant})\)
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
\( t_{(c30)} \) - time in minutes relative to challenge (e.g. 30 minutes after insulin injection)
TMB - tetramethylbenzidine
Zn - zinc