BRIEF COMMUNICATION: Insulin response to a glucose tolerance test in 18-month-old pregnant heifers as a predictor of subsequent herd survival

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Introduction

A decline in dairy cow fertility in the USA and in the UK (Beam & Butler 1999; Royal et al. 2000) has also been noted in New Zealand. Calving rate, the proportion of cows re-calving within the first 42 days of the subsequent seasonal calving period, in New Zealand decreased from approximately 70% in 1991 to 60% in 2004 (Harris et al. 2006). There has been no further deterioration in subsequent years.

A number of studies have indicated that circulating insulin concentrations influence reproductive performance of dairy cows (Garnsworthy et al. 2009; Wathes et al. 2011). The objective of this paper was to investigate the relationship between response parameters to a glucose tolerance test (GTT) administered to pregnant Friesian-Jersey crossbred heifers and their subsequent survival in the herd, especially those aspects associated with fertility.

Materials and methods

The experimental procedure was conducted at the AgResearch, Tokanui Research Farm, near Te Awamutu, New Zealand, in January and February of 2002 (Cohort 1) and 2003 (Cohort 2). All treatments and measurements were approved by the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

Experimental design and animal management

Six F1 bulls of high genetic merit with an average breeding worth of 155, that were reciprocal crosses of purebred or ⅞ Holstein-Friesian and Jersey mixed age animals, were used to artificially inseminate first cross cows selected from commercial dairy herds. The second cross heifer calves that were used as challenge animals, were born in 2000 (Cohort 1) and 2001 (Cohort 2) and had a breeding worth of 139 (Spelman et al. 2004). From weaning until the GTT was carried out, all heifers were managed on one farm following a heifer rearing protocol based on commercial New Zealand dairy farm practice and rotationally grazed on a mixed ryegrass/white clover pasture. Management of the lactating cows in the herd from which the heifers were derived, including feeding and reproductive management, has been described previously (McNaughton et al. 2007).

The heifers in Cohort 1 were divided into 12 challenge groups of 20 to 25 animals while the heifers in Cohort 2 were divided into 14 groups of 20 to 25 animals. The first group in Cohort 1 was challenged on 10 January and the last group on 19 February 2002, the equivalent dates for Cohort 2 were 7 January and 20 February 2003. Two groups were challenged per week. The groups were balanced for sire, live weight and date of birth. At the time of the GTT, the heifers weighed 258 to 451 kg and were 507 to 605 days of age.

Intravenous glucose tolerance test

The day before the GTT, heifers had a catheter (Becton-Dickinson, Franklin Lakes, New Jersey, USA. Angiocath™ – 14 Ga) inserted into the jugular vein under local anaesthesia (Lignocaine 2%). Feed was withheld for 16 hours before the GTT commenced at 1200 hours. A 40% glucose solution (Bomac Laboratories Limited, Auckland, New Zealand) was given I/V at a dose of 0.3 g glucose per kg of live weight via the catheter. Blood samples were collected at -20, -10 and -5 minutes before and 2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after injection of the glucose bolus, into heparinized 10 mL blood collection tubes and immediately placed on ice. The catheters were flushed after each blood sample with 5 mL sterile saline containing 50 IU/mL heparin. Plasma was separated by centrifugation and stored at -80°C until analysis.

Hormone assays

Hormone and metabolite assays were carried out at Alpha Scientific Limited, Hamilton, New Zealand. Plasma glucose concentrations were determined using the Roche Glucose HK liquid assay kit (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). The inter-assay coefficient of variation was 3%. Insulin concentrations were measured in a chemiluminescence assay as per the method given in the IMMULITE® Insulin (PILKIN-6, 2002-07-18) kit insert (Diagnostic Products Corporation, Los Angeles, California, USA). The interassay coefficient of variation was 5.2%.

Other measurements

Reasons for individual cows being removed from the herd were recorded by farm staff at the time of the event. These data were classified into five categories on the basis of whether the heifers were removed on the grounds of metabolic or infectious disease, not pregnant after maiden heifer mating and did not have

Table 1 Response variables to an intravenous glucose tolerance test in 18-month-old pregnant Friesian Jersey crossbred heifers.

Response	Number of heifers	Mean ± standard error of mean
Glucose baseline concentration (mmol/L)	649	4.33 ± 0.01
Glucose maximum concentration (mmol/L)	649	19.44 ± 0.07
Glucose area under curve between 0 and 120 minutes (mmol/L.min)	624	452 ± 3
Glucose fractional decay (t = time to 45) /min	649	-0.020 ± 0.0001
Insulin baseline concentration (mIU/L)	649	2.93 ± 0.07
Insulin maximum concentration (mIU/L)	649	133 ± 3
Insulin area under curve between 0 and 120 minutes (mIU/L.min)	571	3,720 ± 85
Insulin fractional decay (t = time to 45) /min)	649	-0.037 ± 0.0005

Table 2 Mean ± standard error of mean for insulin maximum concentration in plasma and insulin area under the curve between 0 and 120 minutes (adjusted for cohort and experimental group) following an intravenous glucose tolerance test, conducted in 638 18-month-old, pregnant, Friesian Jersey crossbred heifers. Data are grouped by subsequent herd survival categories and tested against those present in the herd at the end of the trial. Disease/illness = Culled or died due to metabolic or infectious disease, Empty0 = Not pregnant after maiden heifer mating, did not have any lactations; Empty1 = Not pregnant at the end of Lactation 1; Empty2 = Not pregnant at the end of Lactation 2; Empty3 = Not pregnant at the end of Lactation 3; Empty4 = Not pregnant at the end of Lactation 4: Present = Completed four lactations and not culled for being non-pregnant at the end of Lactation 4. P values in bold type indicates significance at P <0.05. P value in italic type indicates significance between P = 0.05 and P = 0.10.

Herd survival category	Number of heifers	Maximum insulin response (mIU/L)		Insulin area under curve between 0 and 120 minutes (mIU/L)	
		Mean	P value	Mean	P value
Disease/illness	27	101 ± 13	**0.01**	3243 ± 401	*0.06*
Empty0	28	135 ± 13	0.98	3661 ± 389	0.95
Empty1	20	103 ± 15	**0.04**	2640 ± 463	**0.03**
Empty2	36	132 ± 10	0.80	3436 ± 337	0.99
Empty3	27	122 ± 13	0.31	3824 ± 424	0.84
Empty4	60	129 ± 9	0.52	3808 ± 270	0.35
Present	440	135 ± 3		3721 ± 100	

any lactations, not pregnant at the end of Lactation 1, not pregnant at the end of Lactation 2, not pregnant at the end of Lactation 3, not pregnant at the end of Lactation 4 or Present having completed four lactations and not culled for being non-pregnant at the end of Lactation 4.

Calculations and statistical analyses

Plasma glucose and insulin kinetics during the GTT were calculated using established methods (Kaneko 1997; Lemosquet et al. 1997). Area under the insulin response curve was not accurately assessed in about 10% of animals and these data were excluded.

Data from challenge groups were pooled within 7–8 day periods (2–3 groups per period) as animal numbers were relatively small per group (n = 20–25). Data were adjusted for the fixed effect of these larger groups, nested within cohort, prior to further data analysis. Analysis of variance was carried out on the insulin and glucose parameters generated from the glucose tolerance test described in Table 1. Statistical analyses were carried out in JMP 10.0 (SAS 2012).

Results

Administration of glucose caused a four-fold increase in blood glucose concentration from baseline to peak response (Table 1). Peak glucose concentrations were measured at 2 minutes after the completion of administering the glucose bolus in all animals. Glucose concentration rapidly declined following the peak with 11% of the heifers having regained their basal concentration by 90 minutes after administration of the bolus. The glucose bolus caused, on average, a 45-fold increase in circulating insulin (Table 1). Maximum plasma insulin concentrations were reached 10 to 20 minutes after administration of the glucose bolus, followed by a period of linear decline.

The mean maximum insulin concentration in plasma for animals that died or were culled for metabolic or infectious disease was lower than for animals that had not been culled from the herd at the end of the fourth lactation (Table 2). The maximum insulin response was also significantly lower in animals that were culled for infertility during the first

lactation. The area under the insulin response curve was also lower in the empty group in Lactation 1 but not for the Disease/illness group. Insulin baseline and peak milk yield were not significantly different between the six survival categories and there were no significant differences between the six survival categories for any of the glucose parameters (KJ Hutchinson, Unpublished data).

Discussion

This study demonstrates that differences in fertility and susceptibility to metabolic or infectious disease, to the end of their fourth lactation, were associated, in part, with insulin response to a GTT administered when the heifer was 18-month-old. Less insulin was released in response to glucose in animals that were not pregnant at the end of the first lactation compared with animals that were still in the herd at the end of their fourth lactation.

Heifers releasing more insulin in response to a GTT may maintain a higher circulating concentration of insulin during lactation and so lessen the severity of glucose deprivation to peripheral tissues. Alternatively, maintenance of higher circulating insulin concentration may have a direct impact on reproductive function. Support for a role for insulin in reproduction has recently been demonstrated in a study indicating that cows in severe negative energy balance experienced changes in insulin growth factor and insulin signalling pathways (Wathes et al. 2011).

The effects of insulin response to a GTT on cow survival in the herd were small but statistically significant. Further the data fits with some of the known endocrinology modulating reproductive function in cattle. A better understanding of the relationship between insulin, fertility and disease processes may facilitate improvement in animal health and survival in dairy herds.

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