Evaluation of a model that estimates insulin sensitivity in dairy cows

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ABSTRACT

Glucose challenge data was used to model parameters describing the dynamics of the glucose-insulin system such as glucose effectiveness (S_G), acute insulin response to glucose (AIR_g) and insulin sensitivity (S_I) in dairy cows. The relationship of these parameters with post-partum anovulatory interval (PPAI) was also determined. Twenty-five HF cows of New Zealand (NZ; n=13) and Overseas (OS; n=12) origin, were fed either pasture (n=12) or a total mixed ration (TMR; n=13) in a 2x2 factorial design. At two weeks postpartum, they were challenged intravenously with 300 mg of glucose/kg live weight. There was no effect of diet on S_G (P=0.302), S_I (P=0.17) or AIR_g (P=0.093) despite TMR cows of both genotypes having higher values. There was no effect of genotype on S_G (P=0.234), S_I (P=0.742) or AIR_g (P=0.385). Correlations of these parameters with PPAI were not statistically significant: S_I (r^2=0.087, P=0.310), S_G (r^2 = 0.001, P=0.962) and AIR_g (r^2=0.036, P=0.320). Use of this model demonstrated that insulin response parameters were not closely associated with PPAI.

Key words: insulin sensitivity; glucose challenge.

INTRODUCTION

North American and Dutch Holstein-Friesian (HF) cattle have undergone genetic selection for increased milk production, however, at the same time, reproductive efficiency has decreased (Verkerk *et al.,* 2000). One possible cause of the reduced fertility is the increasing metabolic demand placed on these cows by increasing milk yield. Overseas Holstein Friesian cows (OS-HF) also have a greater propensity to mobilise body reserves during early lactation than New Zealand HF cows (NZ-HF, Kolver *et al.,* 2001). This has implications for the New Zealand dairy industry because the OS-HF cows have high milk production but a reduced ability to recover body fat deposits during lactation (Roche *et al*., 2006). In a seasonal, pasture-based system this can have detrimental effects because some cows do not recover enough body reserves before the next lactation.

Several studies have indicated that certain differences between cattle in processes of energy and protein metabolism are associated with differences in genetic merit for milk production (Bryant & Trigg, 1981; Davey *et al.,* 1983). Differences in energy metabolism could explain the variation in energy partitioning and may also provide an insight into fertility issues, via an influence on postpartum anovulatory interval (PPAI). Insulin sensitivity may also play a role in the regulation of genotype-related differences in nutrient partitioning. Insulin is a major regulator of adipose tissue metabolism during lactation. Glucose is partitioned away from uptake by adipose and muscle cells, and towards uptake by the mammary gland, by a decrease in the sensitivity of adipose and muscle tissue to insulin in the lactating animal (Cronjé, 2000). A better understanding of the genetic and nutritional basis of hormonal regulation of nutrient partitioning will be important to develop nutritional strategies to meet the requirements of lactating cows with different genotypes, and to find ways to improve reproductive efficiency.

In this paper we describe the use of the MINMOD Millennium (Boston *et al*., 2003) programme in dairy cows. This programme enables the estimations of various indices of glucose/insulin dynamics, for example, glucose effectiveness, insulin sensitivity and pancreatic responsivity, which have potential applications in the field of energy metabolism in ruminants. In addition, the relationships of the above-mentioned indices with the resumption of ovarian activity postpartum was examined.

Metabolic and endocrine data from this trial have been published in previous proceedings (Kolver *et al*., 2001; Chagas *et al*., 2003). Briefly, cows of both NZ and OS genotypes were fed pasture (grass) or a total mixed ration (TMR) diet. Following a glucose challenge, the NZ TMR cows had higher plasma insulin concentrations than the NZ grass, resulting in a significant interaction between diet and genotype in the area under the response curve; but the OS groups had similar patterns (Chagas *et al.*, 2003). This result demonstrated metabolic differences between the genotypes and also diets. Plasma concentrations of glucose and IGF-I were not affected by genotype

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or feed regime (Chagas *et al*., 2003).

The aim of this paper is to model indices of glucose and insulin dynamics to further understand the difference in insulin regulation between genotypes and diets and if this influenced PPAI.

METHODS AND MATERIALS

Experimental design and treatments

The experiment design was described in detail by Kolver *et al.* (2001) and the glucose challenge and measurements have been fully described previously (Chagas *et al.,* 2003). All animal experimentation was performed following approval by the Ruakura Animal Ethics Committee. Briefly, twenty-five HF cows of New Zealand (NZ; n=13) and Overseas (OS; n=12) origin, were fed either pasture (Grass; n=12) or a total mixed ration (TMR; n=13) in a 2x2 factorial design.

The glucose tolerance test procedure and plasma glucose and insulin analyses are reported in Chagas *et al.* 2003. The interval to first ovulation was determined using concentrations of progesterone in milk. This was measured using an RIA kit (Coat-A-Count, DPC, CA, USA; Dieleman & Bevers, 1987). The PPAI was defined as the interval from calving to the first of two consecutive sampling days that progesterone concentrations in milk were > 1 ng/ml indicating ovulation.

Glucose/Insulin model

The glucose and insulin data from the glucose challenge trial described above were used in the computer program called MINMOD Millennium (Boston *et al*., 2003) to estimate the parameters of the "minimal" model of insulin sensitivity (*i.e*. the effect of insulin to accelerate glucose disposal). This is different to the other "minimal" model of the glucose-insulin system (Bergman *et al*., 1981a; Pacini & Bergman, 1986) which describes pancreatic sensitivity or responsivity (*i.e*. the effect of glucose to enhance insulin secretion). In the insulin sensitivity model, the insulin data are used as input, and the parameters are calculated in order to produce a simulated glucose pattern as a result of fitting to the measured glucose data. If the pancreatic sensitivity model is used the converse occurs *i.e*. the glucose is the input, and fitting occurs to the insulin data (Pacini & Bergman, 1986). The parameters themselves or indices derived from the parameters of the "minimal" models can then be used to describe the dynamics of the glucose-insulin system. The parameters and indices of the "minimal" model of insulin sensitivity include:

S_I = Insulin sensitivity: quantifies the capacity of insulin to promote glucose disposal through the GLUT4 receptors and to inhibit the endogenous production of glucose.

S_G = Glucose Effectiveness: capacity of glucose to mediate its own disposal through GLUT1 receptors.

AIR_g = Acute Insulin response to glucose: addresses adequacy of insulin secretion through β-cell function (defined as the area under the plasma insulin curve between 0 – 10 minutes) and as such, is a measure of pancreatic responsivity, which is equivalent to Φ_1, a parameter in the "minimal" model of pancreatic sensitivity.

DI = Disposition Index: equals AIR_g x S_I. It combines information on the individual contributions of insulin sensitivity and pancreatic responsivity to give the speed in which the subject responds to the glucose challenge.

I_b = basal insulin concentration pre challenge.

G_b = basal glucose concentration pre challenge.

Statistical analyses

The parameters obtained from the "minimal" model using MINMOD Millennium were analysed using linear models GenStat 8.2. S_I, AIR_g and DI were analysed after log transformation to stabilise the variance. Conclusions from this analysis are similar to those from the analysis of the non transformed data, so for ease of understanding analysis of the untransformed data is presented. The association between PPAI and the model parameters was obtained using CENSOR procedure in GenStat 8.2. Associations have been calculated ignoring treatment and pooled within treatment.

RESULTS

There was no significant effect of diet or genotype on I_b, G_b, S_G, S_I or DI (Table 1). There tended (P=0.093) to be an effect of diet on AIR_g with TMR cows of both strains having higher values. There was no effect of genotype on AIR_g (P=0.385).

There was no effect of diet or genotype on PPAI (Table 1). Correlations of the model parameters with PPAI were not statistically significant: S_I (r^2=0.087, P=0.310), S_G (r^2=0.001, P=0.962) and AIR_g (r^2=0.036, P=0.320).

Table 1: Comparison of insulin model parameters for New Zealand (NZ) and overseas (OS) Holstein-Friesian cows fed pasture (Grass) or total mixed ration (TMR) diets (values given as means ± standard errors of the means).

	Grass		TMR		P value		
	NZ	**OS**	**NZ**	**OS**	**Diet**	**Genotype**	**Interaction**
S_I $(mU/L)^{-1}min^{-1}$	7.37 ± 2.24	7.12 ± 2.24	6.18 ± 1.89	7.29 ± 2.04	0.801	0.809	0.752
S_G (min^{-1})	0.017± 0.003	0.016± 0.003	0.023± 0.016	0.003± 0.003	0.302	0.234	0.375
AIR_g $(mU\ L^{-1}\ min^{-1})$	328 ± 156	442 ± 156	829 ± 144	461 ± 156	0.093	0.385	0.13
DI	3160 ± 826	3368 ± 826	3257 ± 765	2519 ± 826	0.663	0.731	0.567
I_b $(mU\ L^{-1})$	2.418 ± 0.484	2.708 ± 0.484	3.014 ± 0.448	2.28 ± 0.484	0.831	0.617	0.293
G_b $(mg\ dL^{-1})$	2.783 ± 0.178	2.696 ± 0.178	2.857 ± 0.165	2.742 ± 0.178	0.733	0.566	0.937
PPAI	24.7 ± 6.0	25.8 ± 6.6	29.3 ± 5.6	41.3 ± 6.0	0.216	0.256	0.378

S_I – insulin sensitivity; AIR_g - acute insulin response to glucose; I_b – basal insulin
S_G – glucose effectiveness; DI – disposition index; G_b basal glucose
PPAI – postpartum anovulatory interval

DISCUSSION

Kolver *et al*. (2000) described the difference in energy partitioning between NZ and OS genetics and how this was affected by diet (Kolver *et al*., 2001). As part of a glucose challenge experiment, Chagas *et al*. (2003) analysed glucose and insulin concentrations at each sample time and the area under the response curves. They demonstrated that NZ TMR fed cows compared to NZ and OS Grass fed cows and OS TMR fed cows had higher plasma insulin concentrations, resulting in a significant interaction between diet and genotype in the area under the response curve. To explore the insulin and glucose data further, in the present study we used the "minimal" model for insulin sensitivity of Bergman *et al*. (1979) to describe part of the dynamic response to the glucose challenge. The only comparable parameter between that generated by the model and that described by the challenge data in Chagas *et al*. (2003) is the area under the insulin response curve (AIR_g in the model). However, AIR_g calculates the area for just the first 10 minutes post-challenge (the acute insulin response), whereas the area described in Chagas *et al*. (2003) is the area from 0 to 120 minutes post-challenge (the complete insulin response). Using AIR_g the model did not find a significant interaction between diet and genotype in the area under the response curve, whereas using the complete insulin response Chagas *et al*. (2003) did.

We recognised that, to our knowledge, this is the first application of this "minimal" model to lactating dairy cows. Finegood (1997) found that the parameters derived from the "minimal" model of insulin sensitivity may not be an accurate representation of the physiological system when the model is used on species for which it has not been validated. Most of the studies using the "minimal" model have been performed on human subjects, a significant number have been performed on dogs, and a few on primates, rats, pigs and cats. Validations of the "minimal" model have been done for humans and dogs (Finegood, 1997).

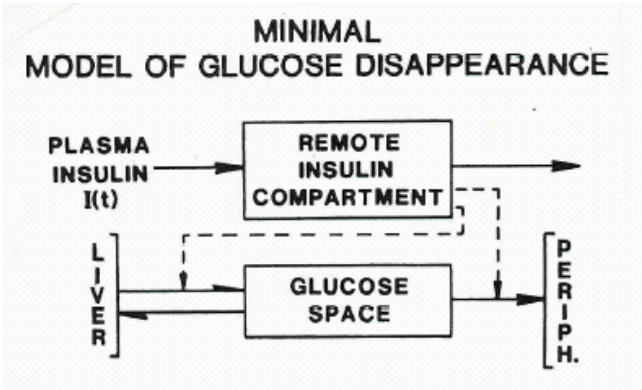
There are other models of glucose/insulin kinetics besides the "minimal" model. These include a model derived by De Gaetano & Arino (2000) which combined the two parts of the "minimal" model (insulin sensitivity and pancreatic responsivity) into a single unified model. Lemosquet & Faverdin (2001) also developed a model that was specifically for use with dairy cows. We chose to use the "minimal" model because, unlike the models of De Gaetano & Arino (2000) and Lemosquet & Faverdin (2001), a suitable computer program incorporating it was readily available, namely MINMOD Millennium (Boston *et al*., 2003).

The first published description of the "minimal" model for insulin sensitivity was given in Bergman *et al.* (1979). Subsequently there have been many papers describing and using both "minimal" models (insulin and pancreatic sensitivity), for example Bergman *et al*. (1981a), Bergman (1989), and Best *et al*. (1996). Pacini & Bergman (1986) incorporated both models into a computer program called MINMOD. The insulin sensitivity part of MINMOD was modified and improved by Boston *et al.* (2003) and is called MINMOD Millennium. "Minimal" means that the model selected was of sufficient complexity to adequately describe the measured FSIGT (frequently sampled intravenous glucose tolerance test) or glucose challenge data, but was of sufficent simplicity to allow estimation of the coefficients or parameters in the model equations for a single subject from a single glucose challenge experiment (Bergman, 1989). The "minimal" model for insulin sensitivity assumes that plasma insulin (I) enters a 'remote' compartment where it is active in accelerating

glucose (G) disappearance into the peripheral tissues and liver, and inhibiting hepatic glucose production, see Figure 1 (Bergman *et al*., 1981b). The model parameters are estimated by fitting the outputted model glucose concentrations to those measured (from the glucose challenge), with the measured time course of insulin (from the glucose challenge) being the input to the model. For more detailed descriptions of the "minimal" model, the reader is referred to the above papers.

Models of glucose-insulin dynamics have been developed and used, because while most of the model parameters can be measured experimentally (*e.g.* by glucose clamp), the methods used are generally difficult to perform, labour intensive, expensive and not without risk to the subject (Pacini & Bergman, 1986). In contrast, models, like the "minimal" model, only involve the frequent sampling of peripheral plasma after an intravenous glucose injection (Bergman *et al*., 1979). Using models though, instead of extensive experimental procedures, means that in order for the models to be of any practical use they first need to be validated by conducting experiments like glucose clamp studies. This is especially true for dairy ruminant animals whose glucose metabolism is different to that of non-ruminants. If the model used in this study is so validated, then it can be more assuredly used to elucidate glucose/insulin kinetics for future work wth dairy cows.

Figure 1: Graphical description of the minimal model of glucose disappearance from Bergman *et al.* (1981b). Plasma insulin, I(t), enters a "remote compartment" where it is active in accelerating glucose disappearance into the periphery and liver, and inhibiting hepatic glucose production.



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