SHORT COMMUNICATION

Insulin responses during glucose tolerance tests in steers with increasing Wagyu genetic influence

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Mir, P. S., Mears, G. J., Ross, C. M., Husar, S. D., Robertson, W. M., Jones, S. D. M. and Mir, Z. 1998. **Insulin response during glucose tolerance tests in cattle with increasing Wagyu genetic influence**. Can. J. Anim. Sci. **78**: 233–235. Intravenous glucose tolerance tests (IVGTT) were conducted in 18 steers with 0, 50 and 75% Wagyu genetic influence. Glucose clearances were similar for all steers. Plasma insulin concentrations (basal, 5 and 10 min post-infusion) were higher ($P < 0.05$) in 0% Wagyu steers (2.57, 7.36, 9.68 ng mL^{-1}) relative to 50% Wagyu (1.17, 2.59, 5.34 ng mL^{-1}) or 75% Wagyu (0.99, 2.78, 5.00 ng mL^{-1}). A correlation coefficient of 0.71 ($P = 0.005$; $n = 15$) between marbling score of carcasses and plasma glucose concentration 90 min after glucose infusion suggests, possible associations among the propensity of cattle with Wagyu genetic influence to marble, mechanisms of glucose utilization and nature of the insulin response to circulating glucose.

Key words: Plasma glucose, insulin, marbling, Wagyu

Mir, P. S., Mears, G. J., Ross, C. M., Husar, S. D., Robertson, W. M., Jones, S. D. M. et Mir, Z. 1998. **Réponse à l'insuline observée durant les tests de tolérance au glucose chez des bouvillons de proportion croissante d'ascendance Wagyu**. Can. J. Anim. Sci. **78**: 233–235. Des tests de tolérance au glucose administré par voie intraveineuse (TGIV) ont été conduits sur 18 bouvillons possédant 0, 50 et 75 % d'ascendance Wagyu. Les taux de clairance de glucose étaient les mêmes dans les trois groupes. Les concentrations plasmatiques d'insuline basale ainsi qu'à 5 et 10 min après infusion étaient plus élevées ($P < 0,05$) chez les sujets sans sang Wagyu, soit respectivement 2,57; 7,36 et 9,68 ng mL^{-1} que chez ceux à 50 % (1,17; 2,59 et 5,34 ng mL^{-1})ou 75 % d'ascendance Wagyu (0,99; 2,78 et 5,00 ng mL^{-1}). Le coefficient de corrélation de 0,71 ($P = 0,005$, $n = 15$) obtenu entre l'indice de persillé des carcasses et les concentrations plasmatiques de glucose 90 min après l'infusion de glucose soulève la possibilité d'une association entre l'aptitude des bovins à ascendance Wagyu à produire une viande persillée, les mécanismes d'utilisation du glucose et la nature de la réponse insulinique au glucose circulant.

Mots clés: Glucose plasmatique, insuline, persillé, Wagyu

The most distinguishing characteristic of the Wagyu breed of beef cattle is that their carcasses have high amounts of intramuscular fat and thus low marbling scores (Mir et al. 1998; Lunt et al. 1993). Adiposity, and to a lesser extent, fat content of the Longissimus thoracis muscle of steers (from Hereford × Angus cows bred with either Angus or Charolais sires) was reported to be positively correlated to circulating concentrations of insulin in plasma samples collected a week prior to slaughter (Trenkle and Topel 1978). Plasma insulin concentrations appear to be associated with adiposity of animals and the IVGTT has been suggested as a tool for selection of sheep with less adipose tissue (Francis et al. 1994). The objective of the present study was to determine whether plasma insulin or glucose concentrations during the IVGTT were correlated to either marbling score of their carcasses or solvent-extractable fat content of the Longissimus thoracis muscle. This objective was used to test the hypothesis that differences exist between the nature of the insulin response to glucose load in cattle with the ability to deposit high amounts of intramuscular fat relative to those less able to deposit fat in the same depot.

Three groups of six steers each of 0% Wagyu genetic influence (European and British crossbred), 50% Wagyu influence (Wagyu × Angus) and 75% Wagyu influence (steers were from either Wagyu × Angus or Wagyu × European composite cows bred with Wagyu semen). Steers were housed and cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993). Steers were individually housed and were fed a backgrounding diet consisting of 35% rolled barley grain and 65% barley silage with supplements of protein and vitamin/mineral mixture (white salt, 56.7 kg; dicalcium phosphate, 15.0 kg; limestone, 15.0 kg; dynamate, 10.0 kg; zinc sulphate, 1.3 kg; manganese sulphate, 1.5 kg; copper sulphate, 492 g; EDDI (80%), 7.8 g; sodium selenite, 5.5 g; cobalt sulfate, 6.0 g; and vitamins A (10 million IU kg^{-1}), D (1 million IU kg^{-1}), and E (100 000 IU kg^{-1}). Feed was withheld 24 h prior to conducting the test for each steer. The IVGTT were conducted in steers (341.9 ± 48.6 kg) when their ages ranged between 333 and 374 d. The IVGTT followed the procedure described by Wastney et al. (1982) with some modifications.

Abbreviations: G90, plasma glucose concentrations 90 min after intravenous administration of glucose; **IVGTT**, intravenous glucose tolerance test(s); **RIIC**, rate of increase in insulin concentration (ng mL^{-1} min^{-1})

Briefly at 09:00 h steers were catheterized in the left jugular vein and two blood samples were obtained 5 and 2 min prior to infusion of glucose. Glucose was infused as a 50% solution in sterile physiological saline via syringe and needle in the right jugular vein at 0.3 g glucose kg^{-1} body weight. Blood samples were obtained from the left jugular vein 5, 10, 20, 25, 30, 35, 45, 60, 90 and 120 min after glucose infusion. Blood samples were collected in heparinized evacuated tubes and stored on ice until plasma was harvested by centrifugation at 1500 × *g* for 15 min. Glucose and insulin concentrations in plasma were determined by the glucose oxidase test (Sigma kit No. 510 DA, Mississauga, ON) and by double antibody radioimmunoassay (Mears 1993), respectively. Age at slaughter for 0, 50 and 75% Wagyu steers was 447, 511 and 538 ± 7.3 d and liveweight was 515, 530 and 469 ± 12 kg, respectively. Carcasses were graded 24 h after slaughter and the marbling score was determined based on a 10-point inverse scoring system (10 = practically devoid and 1 = abundant; Mir et al. 1998). Fat content of the Longissimus thoracis muscle was determined by extraction with petroleum ether (Mir et al. 1998).

Data from two animals had to be eliminated because of problems with glucose infusion and another animal died prior to slaughter and was removed from the data set. Thus the numbers of steers from each of 0, 50 and 75% Wagyu influence groups were 4, 6 and 5, respectively. Data were statistically analyzed as a completely randomized designed experiment to determine variance (General Linear Models, SAS Institute, Inc. 1994), and differences among means by applying the protected least significant difference procedure, for various parameters for the animals with 0, 50 and 75% Wagyu genetic influence. Correlation coefficients between marbling score and plasma concentrations of glucose and insulin at the various sampling times were determined and predictability of marbling score and fat content of muscle from the linear and square terms of concentrations of glucose and insulin were determined using multiple regression (SAS Institute, Inc. 1994).

Marbling scores for carcasses from 0, 50 and 75% Wagyu steers were 7.5 ± 0.54, 5.6 ± 0.49 and 6.2 ± 0.49. Carcasses from the 50% Wagyu group had higher ($P < 0.05$) levels of marbling fat than those from the 0% Wagyu group. However, the 75% Wagyu group had similar ($P > 0.05$) marbling fat compared with the other two groups. Similarly, the chemical fat content of the Longissimus thoracis of the 50% group was the highest (300 ± 38.3 mg g^{-1}), the 0% Wagyu was the lowest (178 ± 27.1 mg g^{-1}) and the 75% Wagyu was intermediate (230 ± 24.2 mg g^{-1}). The marbling and chemical fat content of muscle samples from 50% Wagyu steers was greater ($P < 0.05$) than that of 0% Wagyu steers but similar to that of 75% Wagyu steers. The 50% Wagyu steers were Wagyu and Angus crossbreds. Angus cattle have the potential to marble substantially (Lunt et al. 1993) and may be the reason for higher marbling fat observed in the 50% Wagyu steers relative to the 75% Wagyu steers which contained 25% genetic influences from either Angus or European breeds.

In order to evaluate the relationship of marbling potential to biochemical parameters the plasma concentrations of glucose and insulin were determined. Fasted plasma glucose concentrations were similar ($P > 0.05$) for all steers and ranged between 4.5 and 6.7 mM (Fig. 1a). Plasma glucose concentrations after glucose infusion were not different among steers with different percentages of Wagyu genetic influence. The glucose concentrations were comparable to results presented by Bigner et al. (1996) for Jersey cows (seven cows, rotated through three treatments) when provided with one of three electrolyte formulae. Despite the similarity in glucose response in all three types of steers, differences in the insulin responses to glucose challenge were observed under the conditions of the experiment (Fig. 1b). Plasma insulin concentrations prior to glucose challenge, and 5 and 10 min after infusion were higher ($P < 0.05$) for the 0% Wagyu steers than for either 50 or 75% Wagyu steers. Time to attain maximum insulin concentration (31.7 ± 7.8 min), area under the insulin response curve (851 ± 148 mm^2) and rate of increase in insulin concentration (RIIC; 0.352 ± 0.079 ng mL^{-1} min^{-1}) were not different for the three types of cattle.

Plasma insulin concentrations prior to infusion were not correlated with chemical fat in the Longissimus thoracis. This differs from the positive correlation between insulin concentrations and muscle fat by Trenkle and Topel (1978). However, insulin concentrations at 20 min ($r = 0.51$, $P = 0.103$, $n = 11$), 30 min ($r = 0.60$, $P = 0.05$, $n = 11$) and 35 min ($r = 0.57$, $P = 0.07$, $n = 11$) after glucose infusion tended to be positively correlated to chemical fat content of the muscle. Muscle chemical fat content and RIIC were also correlated ($r = 0.63$, $P = 0.04$, $n = 11$). However, a negative trend was observed for the correlation between marbling score and insulin concentrations at 20 min after glucose infusion ($r = –0.47$, $P = 0.09$, $n = 15$).

Even though differences in plasma glucose concentrations were not noted for the three types of steers, plasma glucose concentrations from 15 to 120 min post-glucose infusion either tended ($P < 0.1$) to be, or were significantly correlated with marbling score, with the coefficient being greatest for G90 ($r = 0.71$, $P = 0.005$, $n = 15$). Similarly, glucose concentrations from 60 to 120 min post glucose infusion were correlated ($P < 0.05$) to chemical fat content of Longissimus thoracis muscle with the coefficient being greatest for the G90 ($r = –0.77$, $P = 0.006$, $n = 11$). Regression analysis for the dependent variables marbling score and fat content of Longissimus thoracis muscle, indicated that the equations were:

Marbling score = –2.2439 (± 1.917) + 0.8122 G90 (± 0.234); $r^2 = 0.5013$, $P = 0.0046$, $n = 15$

Fat content of Longissimus thoracis = 449 (± 84) – 37.6 G90 (± 9.2) + 199 RIIC (± 66.1); $R^2 = 0.806$, $P = 0.0014$, $n = 11$

Inclusion of the square terms for G90 or RIIC did not enhance the R^2 indicating that the relationship among the factors was not quadratic.

The lower basal plasma insulin concentrations in Wagyu crossbred steers was not expected considering the low marbling scores and the high fat content of the Longissimus thoracis

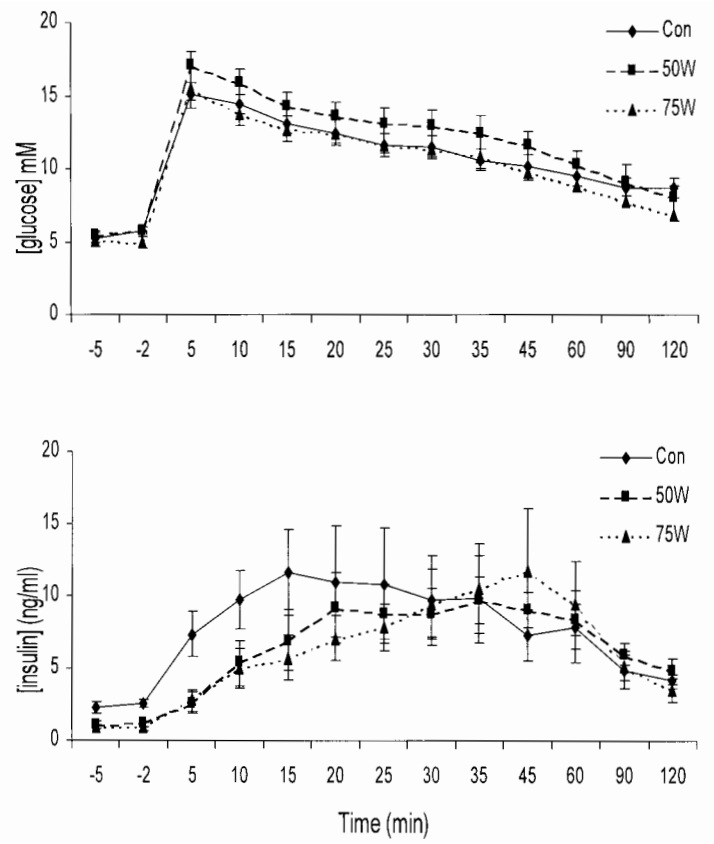

Fig. 1. Mean (± SE) plasma glucose (A) and insulin (B) concentration during intravenous glucose tolerance tests in 0% Wagyu (con), 50% Wagyu (50W) and 75% Wagyu (75W) steers.

of these steers. Trenkle and Topel (1978) showed that plasma insulin was positively correlated with fat deposition in this muscle of steers. In the present study a positive correlation between chemical fat content of muscle and plasma insulin concentration after glucose challenge was observed which supports the correlation found by Trenkle and Topel (1978). Since plasma insulin concentration is the sum of secretion and clearance rates (Trenkle 1976) the lower basal insulin levels in Wagyu crossbred steers may reflect either a lower insulin secretion or a higher insulin turnover in these steers relative to the 0% Wagyu steers. The high marbling fat in 50 and 75% Wagyu cattle and the low basal insulin concentrations suggests the involvement of an insulin independent system for fat synthesis and accumulation in muscle. The operative system involved in deposition of intramuscular fat needs resolution with further work.

The results of this initial study indicate that insulin response to glucose challenge could have a predictive function for determining cattle with the potential to marble extensively. However, the applicability of a tool such as IVGTT for predicting marbling in cattle needs further work.

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