

Tissue responsiveness and sensitivity to insulin in sheep fed plantain and orchardgrass and exposed to cold

H. SANO¹

Y. TAMURA²

A. SHIGA¹

¹Faculty of Agriculture
Iwate University
Morioka 020-8550, Japan
email: sano@iwate-u.ac.jp

²Tohoku National Agricultural Experiment
Station
Morioka 020-0123, Japan

Abstract To assess the effect on insulin action of feeding a forage herb to sheep exposed to a cold environment, eight sheep were fed either plantain (PL), a forage herb, or orchardgrass (OR), a forage. A hyperinsulinaemic euglycaemic clamp procedure was applied to determine tissue responsiveness and sensitivity to insulin in both the thermoneutral (20°C) and cold (0–4°C) environments. For the glucose clamp procedure, insulin was infused over four sequential 2-h periods at rates of 0.64, 1.6, 4.0, and 10 mU kg^{-0.75} body weight min⁻¹ and glucose was infused at variable rates to maintain euglycaemia. The maximal glucose infusion rate (tissue responsiveness to insulin) was greater ($P = 0.04$) for the PL diet than for the OR diet, and was enhanced ($P = 0.001$) during cold exposure. The plasma insulin concentration at half maximal glucose infusion rate (tissue sensitivity to insulin) was influenced by neither diet nor environment. No significant diet by environment interaction was observed for these variables. It is possible that in sheep the forage herb PL enhances insulin action through enhanced tissue responsiveness. Insulin action in response to cold exposure was comparable between the PL and OR diets.

Keywords herb; cold exposure; glucose clamp procedure; insulin; plantain; sheep

INTRODUCTION

The narrow-leaf plantain (PL, *Plantago lanceolata* L.) is a perennial herb. Plantain contains some pharmacological compounds such as aucubin, catalpol, and acteoside (Ishiguro et al. 1982; Nishibe & Murai-Sasahara 1995). Therefore, it may moderate a variety of stresses for ruminants and may improve their performance. Cold exposure is one of the stresses which reduce productivity, and influence digestive and metabolic responses, intermediary metabolism, and the actions of hormones in ruminants (Weekes et al. 1983; Tsuda et al. 1984; Young et al. 1989; Sano et al. 1995). We found that in sheep fed the PL diet and the orchardgrass (OR, *Dactylis glomerata* L.) diet, net blood glucose turnover rate was similar between the diets in both thermoneutral and cold environments (Sano et al. 2002). However, data on the relationship between the PL diet and insulin action in ruminants exposed to cold has not been reported. The objective of this experiment was to compare tissue responsiveness and sensitivity to insulin in sheep fed either the PL or OR diet and exposed to a thermoneutral and cold environment using a hyperinsulinaemic euglycaemic clamp procedure.

MATERIALS AND METHODS

Animals and diets

Eight cross-bred (Corriedale × Suffolk) sheep (five ewes and three rams), aged 2–4 years and weighing 53 ± 3 kg were used. The sheep were surgically prepared under anesthesia with a skin loop enclosing the left carotid artery. Animals were housed in individual metabolic cages in a controlled environment chamber at an air temperature of 20°C. Sheep were shorn leaving less than 10 mm length

of wool just before being moved to the chamber. They were assigned to two dietary treatments, PL hay or OR hay according to sex, age, and body weight (BW). The animals were fed 20 g dry matter of the diets kg^{-1} BW once daily at 1800 h for 5 weeks. Water was available *ad libitum*. The manger was removed the next morning and the diet was not offered again until 1800 h, except on the day of the glucose clamp procedure when the diet was not offered during the blood sampling period. Catheters were fitted to a jugular vein for infusions and to the arterial loop for blood sampling before the initiation of the glucose clamp procedure, and were filled with 38 g litre⁻¹ of trisodium citrate sterile solution. After determinations in the thermoneutral environment were completed, sheep were shorn to less than 10 mm of wool length again and were exposed to a cold environment (0°C from 1000 to 2200 h, 4°C from 2200 to 1000 h) for 5 days. Surgery and blood sampling were carried out according to the guidelines established by the Animal Care Committee of Iwate University.

Table 1 Chemical components and macrominerals of plantain (PL, *Plantago lanceolata* L.) and orchardgrass (OR, *Dactylis glomerata* L.) diets.

Item	PL	OR
Chemical components (g kg ⁻¹ dry matter)		
Crude protein	135	145
Neutral detergent fibre	324	528
Acid detergent fibre	239	285
Crude ash	124	124
Ether extract	15	34
Macrominerals (g kg ⁻¹ dry matter)		
K	37	43
Ca	19	3.1
Mg	1.6	1.6
P	3.4	3.2

Experimental procedures

The hyperinsulinaemic euglycaemic clamp procedure was applied to determine tissue responsiveness and sensitivity to insulin in the thermoneutral environment and on the fifth day of cold exposure (Sano et al. 1992). Insulin (recombinant human insulin, Novo Nordisk, Denmark), diluted in saline solution, was infused over four sequential 2-h periods at rates of 0.64, 1.6, 4.0, and 10 mU $\text{kg}^{-0.75}$ BW min^{-1} via the infusion catheter using a multichannel peristaltic pump (AC-2120, Atto Co. Ltd. Japan). Blood glucose concentrations were determined by an automated glucose analyser immediately after blood sampling (1 ml) at 5-min intervals and were maintained at the preinfusion values by concomitant variable infusion of 200 g litre⁻¹ glucose solution via the infusion catheter using another peristaltic pump during the glucose clamp procedure. Blood samples (3 ml) were taken at 30-min intervals for plasma glucose and insulin determinations. The amount of glucose infused was recorded throughout the 8-h period of insulin infusion. After finishing the insulin infusion, glucose infusion was continued while blood glucose concentrations were monitored for a minimum of 1 h to prevent hypoglycaemia.

Chemical analyses

Chemical components of diets were analysed by the AOAC (1990) method. Potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) contents in the diets were determined by an atomic absorption spectrometer (Z-8100, Hitachi Ltd. Japan). Blood samples were centrifuged at 8000 × g for 10 min at 4°C (RS-18IV, Tomy, Japan), and the plasma was stored at -25°C until further analyses. Concentrations of glucose in blood and plasma were determined with the automated glucose analyser (GLU-1, DKK-TOA Co. Japan). Plasma insulin concentrations were measured with a RIA kit (IRI

Table 2 Dry matter intake (DMI) and weight change in sheep fed plantain (PL, *Plantago lanceolata* L.) and orchardgrass (OR, *Dactylis glomerata* L.) diets in thermoneutral (20°) and cold (0–4°C for 5 days) environments.

Item	PL		OR		SEM	Diet	P value	
	Thermoneutral	Cold	Thermoneutral	Cold			Environ- ment	Inter- action
No. of sheep	4	4	4	4				
DMI (g kg ⁻¹ BW day ⁻¹)	20	20	18	18	0.4			
Weight change (kg day ⁻¹)	0.1	-0.1	0.1	-0.3	0.07	0.56	0.06	0.50

“Eiken”, Eiken Chemical Co. Ltd. Japan) based on a double-antibody method. The sensitivity of the assay was $5.0 \mu\text{U ml}^{-1}$, and intra- and interassay coefficients of variation were 6 and 9%, respectively.

Calculations

Mean values with standard errors of the mean (SEM) are presented. Mean values for glucose infusion rates (GIR) and plasma insulin concentrations (PIC) during the second half of each 2-h insulin infusion rate were calculated (Sano et al. 1992). The maximal glucose infusion rate (GIR_{max}) and plasma insulin concentration at half-maximal glucose infusion rate (ED_{50}) were calculated from the GIR and PIC using logistic regression analysis (Clement et al. 1996). They were used as measures of tissue responsiveness and sensitivity to insulin, respectively (Kahn 1978).

All data were analysed with the MIXED procedure of SAS (1996). The split-plot design was used to test for the effects of diet, environment, and their interaction. The main plot was diet, the subplot was environment and the interaction, and sheep were the block. For insulin infusion rates, the repeated statement was used to analyse the effects of insulin infusion rate and treatment interaction. When the interaction was significant, the least square means statement and Scheffe adjustment were used to compare the values of each insulin infusion rate for each treatment. When no interaction was detected, the pooled data were used for analysis of the least square means statement and Scheffe adjustment. Results were considered significant at the $P < 0.05$ level. A tendency to differ was considered at $P < 0.1$.

RESULTS AND DISCUSSION

Of the chemical compositions and macrominerals in the diets, neutral detergent fibre and ether extract were lower for the PL diet, whereas Ca content was considerably greater for the PL diet than for the OR diet (Table 1) as observed previously (Sano et al. 2002). Six sheep consumed all the diets which were fed, but the other two sheep could not eat all the OR diet (Table 2). The residuals of each of the two sheep for the OR diet were 4 and 1 g kg^{-1} body weight (BW) day^{-1} in both environments. Because the sheep consumed the PL diet in comparable amounts to the OR diet, the PL diet seemed to be palatable to the sheep. Weight change differed little between the diets, and BW tended to be reduced ($P = 0.06$) during cold exposure. Similar results were observed in sheep fed fresh PL and OR diets (Sano et al. 2002).

Insulin infusion rate ($\text{mU kg}^{-0.75}$ of BW min^{-1})			
0.64	1.6	4.0	10

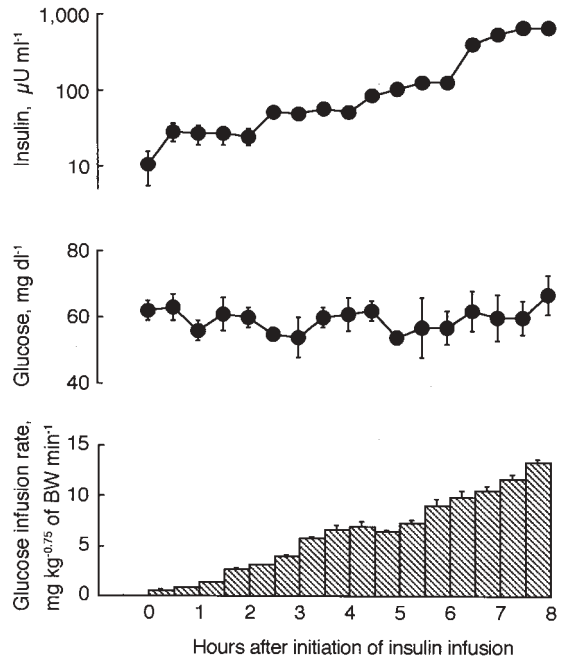


Fig. 1 Plasma insulin and glucose concentrations and glucose infusion rate during the hyperinsulinaemic euglycaemic clamp procedure in sheep ($n = 4$) fed a plantain (*Plantago lanceolata* L.) diet in a thermoneutral environment (20°C).

The tendency toward reduced BW during cold exposure may mainly be due to reduced digestibilities and water intake and enhanced energy expenditure (Kennedy et al. 1976; Kennedy & Milligan 1978; Tsuda et al. 1984) because feed intake was the same in both environments. Crude protein intake was estimated to be similar between the PL and OR diets (Tables 1 and 2). Although gross energy of the diets was not determined in the present experiment, gross energy intake appeared to be comparable between the diets in our previous experiment (Sano et al. 2002).

Because blood glucose concentrations were not maintained at the preinfusion level during insulin infusion in one of the 16 glucose clamp experiments, the data from this study were removed. Therefore, the number of valid experiments was three for the PL diet during cold exposure (Table 3). Plasma insulin concentrations and glucose infusion rates increased with increasing insulin infusion rates (Fig.

1). Plasma insulin and glucose concentrations were virtually constant during the second hour of each 2-h insulin infusion rate. Plasma insulin concentrations did not differ significantly among the treatments, although the concentrations were numerically lower during cold exposure. Glucose infusion rates did not differ between the diets and were enhanced ($P = 0.05$) during cold exposure as observed previously (Weekes et al. 1983; Sano et al. 1992). The GIR_{max} was greater ($P = 0.04$) for the PL diet than for the OR diet, and was greater ($P = 0.001$) during cold exposure than in the thermoneutral environment. The ED_{50} was not significantly influenced by the diet and the environmental temperature. These results indicate that both the PL diet and cold exposure enhanced tissue responsiveness to insulin without changing tissue sensitivity to insulin.

We could not establish whether enhanced tissue responsiveness to insulin was due to the

pharmacological compounds in the PL diet because no pharmacological compound was analysed in the present experiment. Availability of propionate, a major glucose precursor, would be enhanced for the PL diet because propionate concentrations in the rumen were higher for the PL diet than for the OR diet in sheep (Sano et al. 2002). Moreover, Ca content was considerably higher in the PL diet than in the OR diet. Ismail & Namada (2000) reported that, in rats, Ca status influenced insulin secretion and sensitivity. Borycz et al. (1993) demonstrated that, in conscious rats, Ca has a significant role for the hypothalamic-pituitary-adrenal activity. Therefore, it may be possible that these factors would partly explain enhanced tissue responsiveness to insulin for the PL diet. Cold exposure influenced insulin action through enhanced tissue responsiveness for both the diets. Sano et al. (1992) applied a similar glucose clamp procedure to

Table 3 Plasma insulin and glucose concentrations, glucose infusion rates, maximal glucose infusion rate, and plasma insulin concentrations at half maximal glucose infusion rate in the hyperinsulinaemic euglycaemic clamp procedure in sheep fed plantain (PL, *Plantago lanceolata* L.) and orchardgrass (OR, *Dactylis glomerata* L.) diets in thermoneutral (20°) and cold (0–4°C for 5 days) environments. The superscripts with SEM indicate the differences between insulin infusion rates without detecting the insulin infusion rate by treatment interaction. ^{a-d}values in columns with different superscripts differ ($P < 0.05$).

Item	PL		OR		SEM	Diet	P value	
	Thermoneutral	Cold	Thermoneutral	Cold			Environ-ment	Inter-action
No. of sheep	4	3	4	4				
Insulin ($\mu\text{U ml}^{-1}$)								
Preinfusion	10	11	16	14	2 ^c			
0.64	23	25	26	27	2 ^c			
1.6	48	49	54	48	3 ^c	0.95	0.91	0.88
4.0	119	107	121	114	5 ^b			
10	620	504	622	502	29 ^a			
Glucose (mg dl^{-1})								
Preinfusion	62	81	61	72	3			
0.64	59	85	56	68	3			
1.6	58	86	56	70	4	0.08	0.001	0.001
4.0	56	89	57	65	4			
10	62	92	58	68	4			
Glucose infusion rate ($\text{mg kg}^{-0.75} \text{ BW min}^{-1}$)								
0.64	2.6 ^c	5.4 ^d	1.7 ^d	3.8 ^d	0.5			
1.6	6.5 ^b	12.5 ^c	4.6 ^c	8.3 ^c	0.8	0.32	0.05	0.59
4.0	9.0 ^b	19.3 ^b	7.4 ^b	13.7 ^b	1.3			
10	13.4 ^a	25.3 ^a	10.2 ^a	18.4 ^a	1.6			
GIR_{max} * ($\text{mg kg}^{-0.75} \text{ BW min}^{-1}$)	13.4	24.7	10.5	18.6	1.6	0.04	0.001	0.24
ED_{50} † ($\mu\text{U/ml}^{-1}$)	58	51	68	58	4	0.47	0.23	0.70

* GIR_{max} = the maximal glucose infusion rate.

† ED_{50} = the plasma insulin concentration at the half maximal glucose infusion rate.

determine insulin action in sheep and observed that cold exposure enhanced tissue responsiveness to insulin but had little influence on tissue sensitivity to insulin regardless of dietary treatment. Therefore, cold-exposure-accelerated glucose utilisation of the post-receptor site was induced by insulin, without changes in numbers or affinities of receptors. There was no significant diet by environment interaction for the GIR_{max} and ED_{50} , suggesting that the responses of these variables to cold exposure would not be modified by the dietary treatment. However, the possibility could not be excluded that the dietary effect was masked by cold exposure (0–4°C for 5 days) because cold exposure seemed to be a strong stress for the sheep. In conclusion, it is possible that a forage herb, plantain, accelerates insulin action via enhanced tissue responsiveness to insulin. Further studies will be needed to clarify mechanisms of modification due to feeding forage herbs, on endocrine regulation in ruminants.

ACKNOWLEDGMENTS

This study was in part supported by the Research Project for Activation of Joboji Town, Iwate prefecture, Japan. The authors are grateful to K. Taylor, University of Guelph, Canada, for his kind comments on the manuscript.

REFERENCES

- AOAC 1990: Official methods of analysis. 15th ed. Arlington, VA, Association of Official Analytical Chemists.
- Borycz, J.; Bugajski, A. J.; Gadek-Michalska, A.; Bugajski, J. 1993: Calcium channel blockers impair the pituitary-adrenocortical responses to central adrenergic receptors stimulation. *Journal of Physiology and Pharmacology* 44: 161–170.
- Clement, K.; Pueyo, M. E.; Vaxillaire, M.; Rakotoambinina, B.; Thuillier, F.; Passa, P.; Froguel, P.; Robert, J. J.; Velho, G. 1996: Assessment of insulin sensitivity in glucokinase-dependent subjects. *Diabetologia* 39: 82–90.
- Ishiguro, K.; Yamaki, M.; Takagi, S. 1982: Studies on the iridoid related compounds. I. On the antimicrobial activity of aucubigenin and certain iridoid aglycones. *Yakugaku Zasshi* 102: 755–759.
- Ismail, A.; Namada, R. 2000: Impaired glucose tolerance in vitamin D deficiency can be corrected by calcium. *Journal of Nutritional Biochemistry* 11: 170–175.
- Kahn, C. R. 1978: Insulin resistance, insulin insensitivity, and insulin unresponsiveness: A necessary distinction. *Metabolism* 27: 1893–1902.
- Kennedy, P. M.; Milligan, L. P. 1978: Effect of cold exposure on digestion, microbial synthesis and nitrogen transformation in sheep. *British Journal of Nutrition* 39: 105–117.
- Kennedy, P. M.; Christopherson, R. J.; Milligan, L. P. 1976: The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis. *British Journal of Nutrition* 36: 231–242.
- Nishibe, S.; Murai-Sasahara, M. 1995: Bioactive components of plantago herb. *Food & Food Ingredients Journal of Japan* 166: 43–49.
- Sano, H.; Matsunobu, S.; Abe, T.; Terashima, Y. 1992: Combined effects of diet and cold exposure on insulin responsiveness to glucose and tissue responsiveness to insulin in sheep. *Journal of Animal Science* 70: 3514–3520.
- Sano, H.; Nakamura, S.; Kobayashi, S.; Takahashi, H.; Terashima, Y. 1995: Effect of cold exposure on profiles of metabolic and endocrine responses and on responses to feeding and arginine injection in sheep. *Journal of Animal Science* 73: 2054–2062.
- Sano, H.; Tamura, Y.; Shiga, A. 2002: Metabolism and glucose kinetics in sheep fed plantain and orchard grass and exposed to cold. *New Zealand Journal of Agricultural Research* 45: 171–177.
- SAS 1996: SAS/STAT® Software: changes and enhancements through release 6.11. Cary, NC, SAS Institute Inc.
- Tsuda, T.; Ambo, K.; Shoji, Y.; Fujita, M.; Sunagawa, K. 1984: Distribution of energy source expenditure in warm- and cold-exposed sheep. *Canadian Journal of Animal Science* 64 (Suppl.): 265–266.
- Weekes, T. E. C.; Sasaki, Y.; Tsuda, T. 1983: Enhanced responsiveness to insulin in sheep exposed to cold. *American Journal of Physiology* 244: E335–E345.
- Young, B. A.; Walker, B.; Dixon, A. E.; Walker, V. A. 1989: Physiological adaptation to the environment. *Journal of Animal Science* 67: 2426–2432.