Reprod. Nutr. Dev. 46 (2006) 9–18

DOI: 10.1051/rnd:2005064

Original article

Effect of supplemental calcium propionate on insulin action to blood glucose metabolism in adult sheep

Hiroaki SANO*, Tadahisa FUJITA

Department of Agro-bioscience, Faculty of Agriculture, Iwate University, Ueda 3-18-8, Morioka 020-8550, Japan

(Received 10 October 2004; accepted 1 June 2005)

Abstract – An experiment combining a hyperinsulinemic euglycemic clamp procedure of four sequential 2-h periods and an isotope dilution method of [U-^{13}C]glucose determined the effect of supplemental calcium propionate on blood glucose metabolism during insulin and glucose infusions in adult sheep. They were fed lucerne hay cubes and commercial concentrate with and without supplementary calcium propionate (Prop and Cont diets, respectively) in a crossover design for each 21-day period. At the preinfusion period, blood glucose turnover rate (GTR) was greater ($P < 0.05$) for the Prop diet than for the Cont diet. Blood GTR, endogenous glucose production rate (EGPR) and the ratio of EGPR to blood GTR were greater ($P < 0.01$, $P < 0.05$ and $P < 0.05$, respectively) for the Prop diet than for the Cont diet. Blood GTR and glucose infusion rate (GIR) increased ($P < 0.001$) and the ratio of EGPR to blood GTR was reduced ($P < 0.01$) with increased insulin infusion rates. The maximal GIR tended to be ($P < 0.10$) greater for the Prop diet than for the Cont diet but plasma insulin concentration at half maximal GIR did not differ between diets. It is suggested that in adult sheep, dietary propionate supplementation enhances insulin action on glucose metabolism, however, changes in measures of tissue responsiveness and sensitivity were not significant.

calcium propionate / glucose clamp technique / glucose metabolism / insulin / isotope dilution method / sheep

1. INTRODUCTION

In ruminants, glucose utilization differs from non-ruminant animals, because they depend mainly on gluconeogenesis for their glucose requirement. Propionate produced in the rumen is quantitatively the most important precursor for gluconeogenesis [1–3]. Propionate supplementation influences the net flux of glucose in lambs [4] and fat deposition and skeletal muscle growth in wethers [5]. However, the effects of supplementation and intraruminal infusion of propionate on blood glucose kinetics are not persistent in ruminants [6–8]. Intraruminal and intravascular infusions of propionate increase the release of insulin and glucagon [9, 10]. Therefore, it is probable that propionate status in the rumen influences blood glucose metabolism and its endocrine control, and plays an important role in ruminant production. However, little information is available about nutrient metabolism and control, especially in the in vivo studies. A glucose clamp procedure has been used to determine whole body tissue

* Corresponding author: sano@iwate-u.ac.jp

Table I. Composition and metabolizable energy of lucerne hay cubes and commercial concentrate.

	Lucerne hay cubes	Commercial concentrate
Crude protein (% DM)	16.2	17.1
Crude ash (% DM)	9.6	10.2
Ether extract (% DM)	2.1	3.6
Crude fiber (% DM)	27.3	5.3
Nitrogen-free extract (% DM)	44.8	63.8
Metabolizable energy ($MJ \cdot kg^{-1}$ DM)	8.2	12.1

responsiveness and sensitivity to insulin [11]. Weekes et al. [12] applied the glucose clamp procedure combining an isotope dilution method of [6-^{3}H]glucose to determine whole body glucose turnover rate (GTR) and endogenous glucose production rate (EGPR) in addition to tissue responsiveness and sensitivity to insulin in sheep in vivo. It was hypothesized that the propionate supplementation enhances tissue responsiveness and sensitivity to insulin, thus modifying the production of ruminants. Therefore, the present experiment was designed to investigate blood glucose metabolism during the glucose clamp procedure, using the combined experiment which used the isotope dilution method of [U-^{13}C]glucose as a tracer, in adult sheep fed diets supplemented with a physiological level of calcium propionate.

2. MATERIALS AND METHODS

2.1. Animals and diets

Four crossbred (Suffolk × Corriedale) sheep aged 1 to 2 years and weighing 47 ± 1 kg were used. At least 3 months before the experiment, they were surgically prepared under anesthesia with a skin loop enclosing the left carotid artery. The animals were housed in individual pens of the animal room and were shorn to less than 10 mm before the initiation of the experiment. The sheep were allocated to two dietary treatments with a crossover design for 21 days in each experimental period. Basal diets were made up of lucerne hay cubes and commercial concentrate (33% maize, 33% wheat bran, 24% soybean meal, and 10% molasses and minerals). The composition and metabolizable energy (ME) of the basal diets are shown in Table I. The control (Cont) treatment was to feed lucerne hay cubes and concentrate at 17.5 and 4.4 g dry matter (DM)$\cdot kg^{-1}$ body-weight (BW) per day, respectively. The basal diet was estimated to contain 9.0 MJ of ME$\cdot kg^{-1}$ DM [13]. The propionate supplementation (Prop) treatment was the basal diet plus 10 mmol calcium propionate$\cdot kg^{-1}$ BW per day (1.86 g$\cdot kg^{-1}$ BW per day), corresponding to approximately 50% of the daily propionate production in the rumen of sheep [14]. The ME intake was 197 and 228 kJ$\cdot kg^{-1}$ BW for the Cont and Prop diets, respectively. Therefore, the Prop diet was estimated to contain 16% greater ME than the Cont diet [13]. The sheep were fed the diet once daily at 1900 h and consumed it within 1 h. Water was available ad libitum. The sheep were moved to metabolism cages in a controlled environment chamber at an air temperature of 20 ± 1 °C before the experiment. A polyvinyl catheter for infusion was placed in a jugular vein the day before the experiment. A catheter for blood sampling was inserted into the carotid artery of the skin loop in the morning of the experiment. The catheters were maintained by flushing with 3.8% of trisodium citrate sterile solution. The surgery, management and blood sampling were carried out according to the guidelines

established by the Animal Care Committee of the Iwate University.

2.2. Experimental procedures

The experiment, combining the glucose clamp procedure with the isotope dilution method, was carried out on the last day of the 21-day period. At 0900 h of the experimental day, 2 mg of [U-^{13}C]glucose (D-glucose-^{13}C, 99 atom % excess ^{13}C, Isotec Inc., A Matheson, USA Co., USA) dissolved in saline solution was injected into the jugular catheter as a priming dose. Then [U-^{13}C]glucose was continuously infused by a multichannel peristaltic pump (AC-2120, Atto Co. Ltd., Japan) at a rate of 120 μg·min^{-1} through the same catheter for 12 h. Blood samples (3 mL) were taken from the carotid artery catheter immediately before and at 10-min intervals from 180 min to 720 min after the initiation of [U-^{13}C]glucose infusion. They were transferred into centrifuge tubes which contained sodium heparin and were chilled until centrifugation.

The hyperinsulinemic euglycemic clamp procedure was applied to determine blood glucose metabolism in response to insulin infusion [12, 15]. Insulin (64 and 400 U·L^{-1} of saline solution for two lower and two higher infusion rates, respectively; Actrapid monocomponent porcine insulin, Novo Industri, Denmark) was infused over four sequential 2-h periods at rates of 0.64, 1.6, 4.0 and 10 mU·kg^{-1} BW per min by another peristaltic pump through the same jugular catheter during the last 8 h of [U-^{13}C]glucose infusion. The dose and duration of insulin infusion were performed according to the experimental procedures as reported previously [16]. During the glucose clamp procedure, additional blood samples (1 mL) were taken at 5-min intervals for determination of blood glucose concentrations with an automated glucose analyzer within 1 min after collecting blood samples. Glucose solution (20% in distillated water, Daiichi Pharmaceutical Co. Ltd., Japan) was adjustably infused to maintain the preinfusion blood glucose concentrations with a third peristaltic pump into the same jugular venous catheter. The glucose infusion rate (GIR) at 10-min intervals was recorded during the insulin infusion period. After finishing the insulin infusion, glucose infusion was continued while blood glucose concentrations were monitored for a minimum of 1 h to prevent hypoglycemia.

2.3. Analyses

Blood glucose concentrations were determined by the automated glucose analyzer (GLU-1, DKK-TOA Co., Japan) based on the glucose oxidase method. Residual blood samples were centrifuged at 8 500 × *g* for 10 min at 4 ºC, and the plasma was stored at –20 ºC until further analyses. Derivatization of plasma glucose was performed by the procedure of Tserng and Kalhan [17] with slight modifications as described previously [18]. The isotopic abundance of the glucose derivative was determined with a gas chromatographic-mass spectrometric system (DELTA^{plus}, ThermoQuest, Germany). Plasma insulin was determined with a radioimmunoassay kit (IRI "Eiken", Eiken Chemical Co. Ltd., Japan) based on the double antibody radioimmunoassay method. The sensitivity of the assay was 5.0 μU·mL^{-1}, and intra- and interassay coefficients of variance were 6 and 9%, respectively.

2.4. Calculations

Mean values with standard errors of the means (SEM) are given. Blood GTR was calculated according to the equation of Tserng and Kalhan [16] and the equation of nonsteady states by Steele [19] before and after the initiation of the glucose clamp procedure, respectively. The glucose distribution volume and the pool fraction were assumed to be 179 mL·kg^{-1} BW and 0.65 as described by Weekes et al. [12] and Brockman [20], respectively. The EGPR was calculated from GTR minus GIR.

The maximal GIR (GIR_{max}) and plasma insulin concentrations at half-maximal GIR

Table II. Effects of supplemental calcium propionate on body weight, feed intake and basal plasma insulin and blood glucose concentrations and basal blood glucose turnover rate in sheep.

	Cont*	Prop	SEM	Significance
No. of sheep	4	4		
Body weight (kg)	47	46	1	–
Feed intake ($g \cdot kg^{-1}$ BW per day)	22	24	0.4	–
Basal insulin ($\mu U \cdot mL^{-1}$)	13	21	4	0.38
Basal glucose ($mg \cdot dL^{-1}$)	53	53	1	0.82
Basal glucose turnover rate ($mg \cdot kg^{-1}$ BW per min)	3.1	3.8	0.2	< 0.05

* Cont = the basal diet alone; Prop = the basal diet with supplemental calcium propionate; SEM = standard errors of the means.

(ED_{50}) were calculated from mean values of plasma insulin concentrations and GIR during the second half of each 2-h insulin infusion rate using logistic regression analysis [21]. They were used as measures of tissue responsiveness and sensitivity to insulin, respectively [11].

Data were analyzed with the MIXED procedure of SAS [22]. The model statement was used when basal plasma insulin and blood glucose concentrations, basal blood GTR, GIR_{max} and ED_{50} were compared between the Cont and Prop diets. The fixed effect was diet, and the random effect was sheep. The repeated statement was used to test the effects of diet, insulin infusion rate and diet × insulin infusion rate interaction as the fixed effects. The random effect was sheep. All results were considered significant at the $P < 0.05$ level. A tendency to differ was considered at $P < 0.10$. The means statement and Tukey-Kramer adjustment were used to compare values between insulin infusion rates ($P < 0.10$).

3. RESULTS

3.1. Basal states

The sheep consumed all the feed which was offered, therefore, feed intake was greater for the Prop diet than for the Cont diet due to supplemental calcium propionate (Tab. II). The time course of changes in plasma insulin and blood glucose concentrations, and GIR before and during the hyperinsulinemic euglycemic clamp procedure is shown in Figure 1. Blood glucose concentrations were stable before the glucose clamp procedure was commenced in both dietary treatments. At the preinfusion period concentrations of plasma insulin ($P = 0.38$) and blood glucose ($P = 0.82$) did not differ between the Cont and Prop diets. Basal blood GTR was greater ($P < 0.05$) for the Prop diet than for the Cont diet.

3.2. Hyperinsulinemic euglycemic clamp

Plasma insulin concentrations increased ($P < 0.001$) and were stable during the ensuing glucose clamp for the Cont and Prop diets (Fig. 1). Blood glucose concentrations were successfully clamped at preinfusion values during the 8-h glucose clamp period. Plasma insulin and blood glucose concentrations did not differ between the dietary treatments ($P = 0.59$ and 0.89, respectively; Tab. III). Glucose infusion rates did not differ between diets ($P = 0.14$) and increased ($P < 0.001$) as insulin infusion rates increased without the diet × insulin infusion interaction ($P = 0.51$).

Blood GTR during the glucose clamp procedure was higher ($P < 0.01$) for the

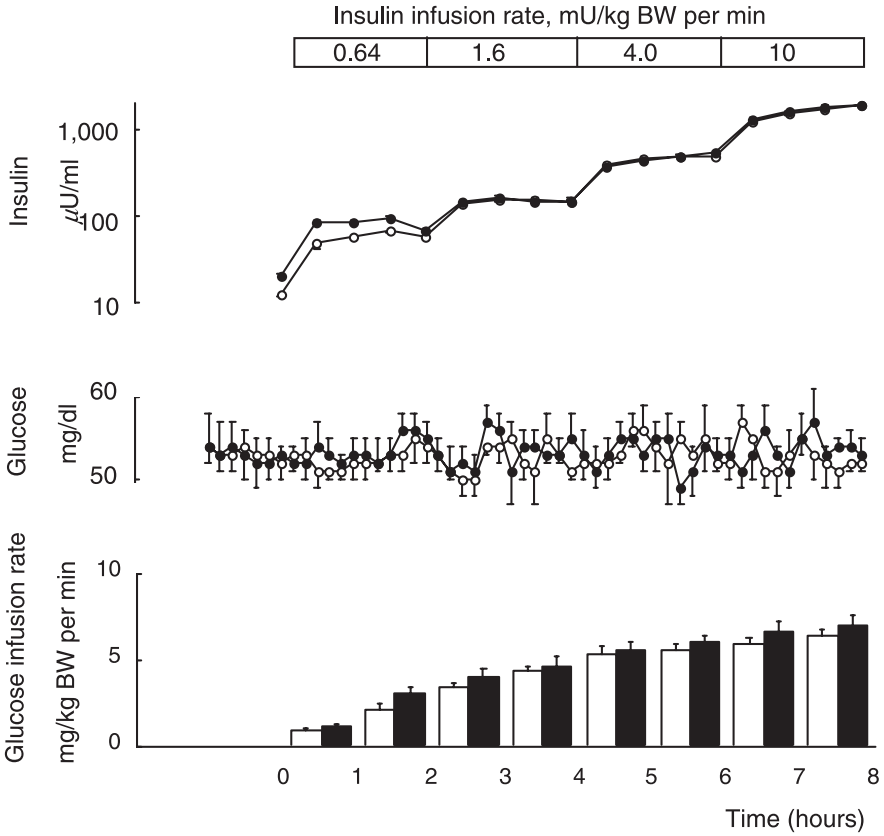

Figure 1. The concentrations of plasma insulin and blood glucose and glucose infusion rates before and during the hyperinsulinemic euglycemic clamp procedure in sheep fed the Cont and Prop diets (open and solid symbols, respectively). Data are expressed as means and SEM.

Prop diet than for the Cont diet. Blood GTR increased ($P < 0.001$) with increasing insulin infusion rates and the diet × insulin infusion rate interaction was not significant ($P = 0.19$). Endogenous glucose production rate was higher ($P < 0.05$) for the Prop diet than for the Cont diet, but was not affected ($P = 0.98$) by insulin infusion rates. The ratio of EGPR to blood GTR was greater ($P < 0.05$) for the Prop diet than for the Cont diet and was reduced ($P < 0.01$) as insulin infusion rates increased. The GIR_{max} tended to be higher ($P < 0.10$) for the Prop diet than for the Cont diet and the ED_{50} did not differ between the diets ($P = 0.37$).

4. DISCUSSION

In the present experiment, nonproductive adult sheep were used. Although insulin action and sensitivity of sheep changed with growth stage as determined by the glucose clamp technique [23], the adult sheep would provide a standard animal model. The sheep were fed lucerne hay cubes and commercial concentrate with or without dietary calcium propionate supplementation at a level of 10 mmol·kg^{-1} BW per day. Although VFA concentrations in the rumen fluid and blood were not determined, the dose of calcium propionate was considered

Table III. Effects of supplemental calcium propionate on plasma insulin and blood glucose concentrations, glucose infusion rate, blood glucose turnover rate and ratio of endogenous glucose production rate to blood glucose turnover rate during the hyperinsulinemic euglycemic clamp procedure in sheep.

	Cont*	Prop	SEM	Significance		
				Diet	Insulin	Diet × Insulin
No. of sheep	4	4				
Insulin ($\mu U \cdot mL^{-1}$)						
Preinfusion	13	21	4[d]			
0.64**	72	83	10[d]			
1.6	153	157	5[c]	0.59	< 0.001	0.96
4.0	487	497	17[b]			
10	1 747	1 796	47[a]			
Glucose ($mg \cdot dL^{-1}$)						
Preinfusion	53	53	1			
0.64	53	54	1			
1.6	53	53	1	0.89	0.93	0.94
4.0	53	53	1			
10	52	54	1			
Glucose infusion rate ($mg \cdot kg^{-1}$ BW per min)						
0.64	2.2	3.1	0.3[d]			
1.6	4.4	4.6	0.3[c]	0.14	< 0.001	0.51
4.0	5.5	6.1	0.2[b]			
10	6.5	7.0	0.3[a]			
Glucose turnover rate ($mg \cdot kg^{-1}$ BW per min)						
Preinfusion	3.1	3.8	0.2[c]			
0.64	4.0	6.5	0.7[bc]			
1.6	6.3	8.3	0.6[ab]	< 0.01	< 0.001	0.19
4.0	7.2	10.3	0.9[a]			
10	7.8	11.4	1.1[a]			
Endogenous glucose production rate ($mg \cdot kg^{-1}$ BW per min)						
0.64	1.9	3.4	0.7			
1.6	1.9	3.7	0.6	< 0.05	0.98	0.84
4.0	1.6	4.2	0.8			
10	1.4	4.4	1.0			
Ratio of endogenous glucose productions rate (%)						
0.64	46	49	5[a]			
1.6	29	43	5[ab]	< 0.05	< 0.01	0.60
4.0	22	38	4[b]			
10	17	35	5[b]			
***GIR_{max} ($mg \cdot kg^{-1}$ BW per min)						
	6.1	6.8	0.3	< 0.10	–	–
† ED_{50} ($\mu U \cdot mL^{-1}$)						
	89	105	10	0.37	–	–

* Cont = the basal diet alone; Prop = the basal diet with supplemental calcium propionate; SEM = standard errors of the means.
** Insulin infusion rate, $mU \cdot kg^{-1}$ BW per min.
*** GIR_{max} = the maximal glucose infusion rate.
† ED_{50} = the plasma insulin concentration at the half maximal glucose infusion rate.
The superscripts with SEM indicate differences ($P < 0.10$) between insulin infusion rates without detecting diet and insulin infusion rate interaction.

to be within the physiological range as described previously [24]. Sano et al. [25] determined the postprandial changes in VFA concentrations in both the rumen fluid and blood in sheep supplemented with the same dose of propionate as in the present experiment. They reported that propionate supplementation increased propionate concentrations in both the rumen fluid and blood after the initiation of feeding but other VFA concentrations did not differ from the diet without propionate supplementation. Similar changes in VFA in response to the calcium propionate supplementation would be expected in the present experiment. Lopez et al. [26] reported that in sheep sustained by intragastric infusions, acetate, propionate, and butyrate were absorbed from the rumen in a concentration-dependent manner. Therefore, propionate absorption for the Prop diet would be greater than for the Cont diet.

Plasma insulin concentrations increased temporarily after the initiation of feeding in sheep fed lucerne hay cubes in a single meal daily [27]. Sano et al. [25] observed that in sheep fed the diet with supplemental calcium propionate once daily, plasma insulin concentrations increased to peaks at 45 min after feeding and decreased thereafter. The insulin response to feeding was greater for the diet with supplemental propionate than for the basal diet alone. Because plasma insulin concentrations for the diet with supplemental propionate returned to the levels of the basal diet alone within several hours after feeding, basal plasma insulin concentrations did not differ between the diets. Moreover, the time course of changes in plasma insulin concentrations over the 8-h period of the glucose clamp procedure was similar between the dietary treatments. It was expected that the doses of insulin infusion were comparable between diets and endogenous insulin release from the pancreas contributed little to the enhanced blood GTR during the glucose clamp procedure.

In the present experiment, the sheep were fed once daily and the isotope dilution method was started at 14 h after feeding. Blood glucose concentrations were stable before the glucose clamp procedure. Therefore, it may be suggested that the blood glucose pool was in a steady state, when the isotope dilution method was performed. Both basal blood GTR and blood GTR during the glucose clamp procedure were enhanced by propionate supplementation. Seal and Parker [8] infused propionic acid into the rumen of growing steers at rates of 0.5 and 1.0 mol·day^{-1}, which were lower doses than in the present experiment when compared on a per BW basis, and reported that glucose irreversible loss rate increased at the higher propionic acid infusion rate (1.0 mol·day^{-1}). On the contrary, Majdoub et al. [7] reported that in growing lambs fed rye grass, the apparent glucose turnover was not modified by intraruminal infusion of propionate (0.55 and 0.91 mol·day^{-1}), despite a numerical increase which was due to one of six animals. The inconsistency of effects of supplemental propionate on blood glucose metabolism may be involved in the dose, duration or method of propionate supplementation, the stage of animals, or feeding managements. Although basal blood GTR was within the ranges of blood GTR as determined by the isotope dilution methods using stable and radio isotopes, blood GTR for the Prop diet seemed to be near the upper margin [12, 18, 28, 29]. Because blood GTR is influenced by the type of diet, energy intake and physiological status [28–30], these factors may be related. Ortigues-Marty et al. [29] reviewed the relationship between glucose turnover rate and ME intake in growing ovines, growing bovines and adult ovines, and suggested that growth stage on glucose turnover rate is probably as important as the effect of animal species.

Our results of the EGPR and the ratio of the EGPR to blood GTR suggested that gluconeogenesis was sustained even while the glucose clamp procedure was performed and they were greater for the Prop diet than for the Cont diet. Seal and Parker [8] found that in growing steers infused with propionate intraruminally, most of the infused

propionate was converted to glucose. Van der Walt [2] reported that in sheep fed lucerne hay at 12-h intervals, 15 to 22% of glucose turnover derived from propionic acid even at the prefeeding period. Plasma glucagon concentrations increased more after the initiation of feeding for the diet supplemented with calcium propionate than for the diet without supplementation [24]. Brockman and Bergman [31] and Brockman and Greer [32] reported that in sheep, glucagon infusion increased hepatic gluconeogenesis from alanine and glutamine but not from propionate. Therefore, it is probable that propionate supplementation increases glucagon secretion and results in enhanced hepatic gluconeogenesis from glucogenic amino acids in addition to propionate.

In the glucose clamp procedure, blood GTR increased with increased insulin infusion rates in a dose-dependent manner as reported previously [15, 33, 34]. This reflected enhanced glucose utilization in insulin dependent tissues such as skeletal muscle and adipose tissue [35]. The ratio of EGPR to blood GTR was reduced to 17 or 35% as insulin infusion rates increased. Weekes et al. [12] reported that in sheep, the ratio of EGPR was reduced to approximately 20%, even when higher insulin infusion rates than the present experiment were applied. Sano et al. [21] obtained a similar ratio to the present experiment in sheep. On the contrary, Janes et al. [15] reported that in sheep, the EGPR was completely suppressed during the hyperinsulinemic euglycemic clamp procedure at 6 $mU \cdot kg^{-1}$ BW per min of insulin infusion. Bergman et al. [33] also reported that in sheep, complete suppression of endogenous glucose appearance rate occurred at insulin concentrations of 50 to 300 $\mu U \cdot mL^{-1}$. Because the EGPR at the higher insulin infusion rates was negative in the above two reports, the isotope dilution methods used or the determination of glucose infusion rates would be involved in the errors.

For calculations of tissue responsiveness and sensitivity to insulin, plasma insulin concentrations and GIR were used. The GIR_{max} and ED_{50} were somewhat higher than those reported previously [12, 33, 34], because the step numbers and rates of insulin infusion and the regression equations differed. However, they were comparable between the Cont and Prop diets. Of the measures of tissue responsiveness and sensitivity, only a tendency was detected in GIR_{max} between the Cont and Prop diets. Sano et al. [21] reported that in sheep the action of insulin on glucose metabolism was enhanced during cold exposure, but the effect of feed restriction was somewhat enhanced. It may be possible that nutritional factors are relatively less effective on insulin action as determined by the glucose clamp procedure.

In conclusion, propionate supplementation to forage rich diets at a level close to maintenance ME requirements has the ability to enhance blood glucose metabolism both before and during the hyperinsulinemic euglucemic clamp procedure in adult sheep. Manipulation of rumen VFA fermentation can modify fat deposition and skeletal muscle growth of sheep [5] through enhanced insulin action in addition to insulin secretion.

ACKNOWLEDGEMENTS

This work was in part supported by Research Grants for Meat and Meat Products from the Ito Foundation (2002). The authors are grateful to K. Taylor, Univ. of Guelph, Canada, for his kind advice on the manuscript.

REFERENCES

[1] Bassett JM. Dietary and gastro-intestinal control of hormones regulating carbohydrate metabolism in ruminants. In: McDonald IW, Warner AI (Eds), Digestion and Metabolism in the Ruminant, University of New England, Armidale 1975, p 383–398.

[2] Van der Walt JG. Volatile fatty acid metabolism in sheep. 3. Diurnal variation in the contribution of ruminal propionic acid production to the whole body glucose turnover of Merino

sheep fed lucerne hay twice daily. Onderstepoort J Vet Res 1978, 45: 125–132.

[3] Tsuda T, Ambo K, Shoji Y, Fujita M, Sunagawa K. Distribution of energy source expenditure in warm- and cold-exposed sheep. Can J Anim Sci 1984, 64 (Suppl): 265–266.

[4] Van Houtert MFJ, Nolan JV, Leng RA. Protein, acetate and propionate for roughage-fed lambs. 2. Nutrient kinetics. Anim Prod 1993, 56: 369–378.

[5] Moloney AP. Growth and carcass composition in sheep offered isoenergetic rations which resulted in different concentrations of ruminal metabolites. Livest Prod Sci 1998, 56: 157–164.

[6] Judson GJ, Leng RA. Studies on the control of gluconeogenesis in sheep: effect of propionate, casein and butyrate infusions. Br J Nutr 1973, 29: 175–195.

[7] Majdoub L, Beylot M, Vermorel M, Ortigues-Marty I. Propionate supplementation did not increase whole body glucose turnover in growing lambs fed rye grass. Reprod Nutr Dev 2003, 43: 357–370.

[8] Seal CJ, Parker DS. Effect of intraruminal propionic acid infusion on metabolism of mesenteric- and portal-drained viscera in growing steers fed a forage diet: I. Volatile fatty acids, glucose and lactate. J Anim Sci 1994, 72: 1325–1334.

[9] De Jong A. Patterns of plasma concentrations of insulin and glucagon after intravascular and intraruminal administration of volatile fatty acids in the goat. J Endocrinol 1982, 92: 357–370.

[10] Sano H, Hattori N, Todome Y, Tsuruoka J, Takahashi H, Terashima Y. Plasma insulin and glucagon responses to intravenous infusion of propionate and their autonomic control in sheep. J Anim Sci 1993, 71: 3414–3422.

[11] Kahn CR. Insulin resistance, insulin insensitivity, and insulin inresponsiveness: A necessary distinction. Metabolism 1978, 27 (Suppl 2): 1893–1902.

[12] Weekes TEC, Sasaki Y, Tsuda T. Enhanced responsiveness to insulin in sheep exposed to cold. Am J Physiol 1983, 244: E335–E345.

[13] NRC. Nutrient Requirements of Sheep. 6th ed, National Academy Press, Washington, DC, 1985.

[14] Froetschel MA, Croom WJ Jr, Gaskins HR, Leonard ES, Whitacre MD. Effects of avoparcin on ruminal propionate production and amino acid degradation in sheep fed high and low fiber diets. J Nutr 1983, 113: 1355–1362.

[15] Janes AN, Weekes TEC, Armstrong DG. Insulin action and glucose metabolism in sheep fed on dried-grass or ground, maize-based diets. Br J Nutr 1985, 54: 459–471.

[16] Sano H, Matsunobu S, Abe T, Terashima Y. Combined effects of diet and cold exposure on insulin responsiveness to glucose and tissue responsiveness to insulin in sheep. J Anim Sci 1992, 70: 3514–3520.

[17] Tserng K-Y, Kalhan SC. Estimation of glucose carbon recycling and glucose turnover with [U-^{13}C]glucose. Am J Physiol 1983, 245: E476–E482.

[18] Sano H, Fujita T, Murakami M, Shiga A. Stimulative effect of epinephrine on glucose production and utilization rates in sheep using a stable isotope. Domest Anim Endocrinol 1996, 13: 445–451.

[19] Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 1959, 82: 420–430.

[20] Brockman RP. Effect of insulin on the utilization of propionate in sheep. Br J Nutr 1990, 64: 95–101.

[21] Sano H, Takebayashi A, Kodama Y, Nakamura K, Ito H, Arino Y, Fujita T, Takahashi H, Ambo K. Effects of feed restriction and cold exposure on glucose metabolism in response to feeding and insulin in sheep. J Anim Sci 1999, 77: 2564–2573.

[22] SAS. SAS/STAT® Software: Changes and enhancements through Release 6.11. SAS Inst. Inc., Cary, NC, 1996.

[23] Sano H, Asano K, Noguchi Y, Yoshimura K, Senshu T, Terashima Y. Insulin responsiveness, action and sensitivity in growing lambs and mature rams. Can J Anim Sci 1996, 76: 203–208.

[24] Sano H, Mori Y, Takahashi H, Terashima Y. Autonomic regulation of plasma insulin, glucagon and growth hormone responses to feeding in sheep fed a diet supplemented with calcium propionate. Can J Anim Sci 1999, 79: 449–456.

[25] Sano H, Terashima Y, Senshu T. Insulin secretory response to feeding in sheep fed a diet supplemented with calcium, potassium and sodium propionate. Jpn J Zootech Sci 1989, 60: 70–77.

[26] Lopez S, Hovell FD, Dijkstra J, France J. Effects of volatile fatty acid supply on their absorption and on water kinetics in the rumen of sheep sustained by intragastric infusions. J Anim Sci 2003, 81: 2609–2616.

[27] Sasaki Y, Hiratsuka H, Ishida M. Effect of cold exposure on insulin response to feeding

in sheep. Can J Anim Sci 1984, 64 (Suppl): 269–270.

[28] Janes AN, Weekes TEC, Armstrong DG. Absorption and metabolism of glucose by the mesenteric-drained viscera of sheep fed on dried-grass or ground, maize-based diets. Br J Nutr 1985, 54: 449–458.

[29] Ortigues-Marty I, Vernet J, Majdoub L. Whole body glucose turnover in growing and non-productive adult ruminants: meta-analysis and review. Reprod Nutr Dev 2003, 43: 371–383.

[30] Evans E, Buchanan-Smith JG. Effects upon glucose metabolism of feeding a low- or high-roughage diet at two levels of intake to sheep. Br J Nutr 1975, 33: 33–44.

[31] Brockman RP, Bergman EN. Effect of glucagon on plasma alanine and glutamine metabolism and hepatic gluconeogenesis in sheep. Am J Physiol 1975, 228: 1628–1633.

[32] Brockman RP, Greer C. Effects of somatostatin and glucagon on the utilization of [2-^{14}C] propionate in glucose production in vivo in sheep. Aust J Biol Sci 1980, 33: 457–464.

[33] Bergman EN, Reulein SS, Corlett RE. Effects of obesity on insulin sensitivity and responsiveness in sheep. Am J Physiol 1989, 257: E772–E781.

[34] Weekes TEC. Influence of experimental hyperthyroidism on insulin action in growing sheep. Metabolism 1992, 41: 246–252.

[35] Weekes TEC. Hormonal control of glucose metabolism. In: Tsuda T, Sasaki Y, Kawashima R (Eds), Physiological Aspects of Digestion and Metabolism in Ruminants, Academic Press, San Diego 1991, p 183–200.