Effect of Cold Exposure on Insulin Responsiveness to Glucose and Tissue Responsiveness to Insulin in Sheep Given *Ad Libitum* Access to Roughage

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Abstract Hyperglycemic clamp and hyperinsulinemic euglycemic clamp techniques were applied to investigate the effect of cold exposure on insulin responsiveness to glucose and tissue responsiveness to insulin in sheep given *ad libitum* access to roughage. Daily dry matter intake was not influenced by cold exposure. In the hyperglycemic clamp experiment, the ratio of plasma insulin increment to glucose infusion rate (insulin responsiveness to glucose) was reduced by cold exposure ($P < 0.05$). In the hyperinsulinemic euglycemic clamp experiment, the glucose infusion rate (tissue responsiveness to insulin) was higher ($P < 0.01$) during cold exposure than in the thermoneutral environment. These results suggest that *ad libitum* access to roughage does not influence reduced insulin responsiveness to glucose and enhanced tissue responsiveness to insulin during cold exposure in sheep whose voluntary intakes did not increase.

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Key words : glucose clamp technique, insulin, sheep, *ad libitum* feeding, cold exposure

Insulin is one of the most important hormones to control nutrient metabolism in both ruminant and nonruminant animals. It has been reported in sheep under some feeding regimens that insulin responsiveness to glucose (glucose-induced insulin secretory response) was reduced and tissue responsiveness to insulin (insulin action to tissue) was enhanced during cold exposure using the glucose clamp technique^{3,11)}. They were also influenced by feeding levels⁸⁾ and type of diet³⁾. Moreover, *ad libitum* roughage intake increases heat production¹⁰⁾, and enhanced heat production may relieve the effect of cold stress. Therefore, we postulated that *ad libitum* roughage intake

might affect insulin responsiveness to glucose and tissue responsiveness to insulin differently from those obtained in cold-exposed sheep fed a diet at maintenance level¹¹⁾ or a mixed diet of concentrate and roughage³⁾. However, no information has been available about insulin responsiveness to glucose and tissue responsiveness to insulin using the glucose clamp technique during cold exposure in sheep given *ad libitum* access to roughage.

The objective of this experiment was to investigate the effect of cold exposure on insulin responsiveness to glucose and tissue responsiveness to insulin using the hyperglycemic clamp and hyperinsulinemic euglycemic

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clamp techniques in sheep given *ad libitum* access to roughage.

Materials and Methods

Animals and Diets: Nine Suffolk shorn rams aged 2 to 3 years and weighing about 50 kg were used. They were surgically prepared with a skin loop enclosing the left carotid artery. Animals were initially kept in metabolism cages in an environment controlled chamber at $20 \pm 1^\circ\text{C}$ for more than a month. After the end of the experiment in the thermoneutral environment, the animals were exposed to 0°C . The experimental diet was chopped orchardgrass hay (moisture 12.2%, crude protein 14.1%, ether extract 3.8%, crude fiber 24.4%, crude ash 8.4% and nitrogen free extract 37.1%), and was given *ad libitum* access to feed. The diet and water were given at 16:00 h. Catheters for infusions and blood sampling were inserted into a jugular vein at least 2 days and into the skin loop enclosing at least 2 hours before the experiment, respectively.

Glucose Clamp: The hyperglycemic clamp experiment was carried out to determine insulin responsiveness to glucose (glucose-induced insulin secretory response) on day 11 of the thermoneutral and cold exposure. The preinfusion blood glucose concentrations were determined three times by a glucose analyzer immediately before the glucose infusion. At 13:00 h glucose solution (20% (W/V)) was infused by a multichannel peristaltic pump (Model AC-2120, Atto Co. Ltd., Tokyo) through the jugular catheter for 2 hours, and blood glucose concentrations were designed to achieve 50 mg/dl above preinfusion levels (mean of three determinations) during the glucose infusion period. Blood samples (1 ml) were taken from the arterial catheter every 5 min and blood glucose concentrations were determined by the glucose analyzer within 1 min after taking each blood sample. Then, the glucose infusion rate was adjusted to main-

tain the desired blood glucose concentrations. Blood samples (3 ml) taken at 10-min intervals were stored in ice-water until centrifugation. The glucose infusion rate of 10-min intervals was recorded throughout the glucose infusion period.

The hyperinsulinemic euglycemic clamp experiment was carried out to determine tissue responsiveness to insulin (insulin action to tissue) on day 14 of both environments. Immediately after the determination of the preinfusion blood glucose concentrations, insulin (400 U/l; Actrapid monocomponent porcine insulin, Novo Industry, Denmark) dissolved in 0.9% sodium chloride and 2.5% potassium chloride was infused continuously by the peristaltic pump at the rate of 6.0 mU/kg/min for 2 hours starting at 13:00 h. Blood glucose concentrations were determined every 5 min, and glucose solution (20%) was infused by other peristaltic pump to maintain the preinfusion blood glucose concentrations. Glucose infused was recorded every 10 min as described in the hyperglycemic clamp experiment.

Analyses: Blood glucose concentrations were determined by the glucose analyzer (Model GLU-1, Erma Optical Works Ltd., Tokyo). In the hyperglycemic clamp experiment residual blood samples were centrifuged at $10,000 \times g$ for 10 min at 4°C , and stored at -20°C until assayed for insulin. Plasma insulin was assayed by radioimmunoassay as described previously⁴¹.

Calculations: The glucose infusion rate and the increase in plasma insulin concentrations over the preinfusion level during the last 40 min of the 2-hour period of the glucose clamp experiment were termed the glucose infusion rate (GIR) and the mean plasma insulin increment (MPII), respectively⁴¹.

Differences between the preinfusion mean and values obtained during glucose or insulin infusions were evaluated by Student's paired *t*-test. Differences between the thermoneutral and cold exposure on parameters of physiologi-

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cal responses, insulin responsiveness to glucose and tissue responsiveness to insulin were analyzed by analysis of variance using the General Linear Model procedure of the Statistical Analysis System⁵⁾.

Results

Physiological Responses: Body weight and dry matter (DM) intake of sheep were unchanged during cold exposure (Table 1). Heart rate increased ($P<0.01$), and respiration rate ($P<0.05$) and rectal temperature ($P<0.05$)

decreased during cold exposure.

Hyperglycemic Clamp: The preinfusion concentrations of blood glucose and plasma insulin were not different between the thermoneutral and cold exposure (Fig. 1 and Table 2). Blood glucose concentrations increased ($P<0.01$) accompanied by glucose infusion, and were almost maintained at 50 mg/dl above the preinfusion levels during the last hour of glucose infusion in both environments. The glucose infusion rates were stable at least for the last 50 min of glucose infusion in both

Table 1. Effect of cold exposure on body weight, dry matter intake, ME intake, respiration rate, heart rate and rectal temperature in sheep given a roughage diet *ad libitum*

	Thermoneutral		Cold exposure		Significance
	Mean	SE	Mean	SE	
Body weight (kg)	51.7	1.2	52.1	1.2	NS
Dry matter intake (kg/day)	1.19	0.09	1.18	0.12	NS
ME intake (Mcal/day) ^a	2.41	0.18	2.39	0.24	NS
Respiration rate (min ⁻¹)	39	6	23	1	*
Heart rate (min ⁻¹)	74	4	100	6	**
Rectal temperature (°C)	39.1	0.1	38.5	0.1	*

^a Calculated

* $P<0.05$

** $P<0.01$

NS=not significant

Table 2. The concentrations of blood glucose and plasma insulin and glucose infusion rate in the hyperglycemic clamp experiment in sheep given a roughage diet *ad libitum*

	Thermoneutral		Cold exposure		Significance
	Mean	SE	Mean	SE	
Basal glucose (mg/dl)	51	2	53	2	NS
Clamped glucose (mg/dl)	103	2	104	1	NS
GIR ^a (mg/kg/min)	3.10	0.36	4.08	0.44	*
Basal insulin (μ U/ml)	28	2	24	3	NS
MPH ^b (μ U/ml)	84	13	71	14	NS
MPH/GIR	28	3	17	1	*

^aGIR=glucose infusion rate during the last 40 min of glucose infusion period.

^bMPH=mean plasma insulin increment during the last 40 min of glucose infusion period.

* $P<0.05$

NS=not significant

Fig. 1. The concentrations of blood glucose and plasma insulin, and glucose infusion rate before and during the hyperglycemic clamp experiment in sheep exposed to the thermoneutral (solid symbols) and cold (open symbols) environments. Data are expressed as mean $\pm$ SE of nine sheep.

environments, although they changed during the initial period. The GIR was higher ($P < 0.05$) during cold exposure than in the thermoneutral environment. Plasma insulin concentrations increased ($P < 0.01$) together with increases in blood glucose concentrations, and were stable during the last hour of glucose infusion in both environments. The MPII tended to be lower during cold exposure than in the thermoneutral environment. The ratio of MPII to GIR was lower ($P < 0.05$) during cold exposure than in the thermoneutral environment.

Hyperinsulinemic Euglycemic Clamp: The preinfusion concentrations of blood glucose were higher ($P < 0.05$) during cold exposure

Fig. 2. The concentrations of blood glucose and glucose infusion rate before and during the hyperinsulinemic euglycemic clamp experiment in sheep exposed to the thermoneutral (solid symbols) and cold (open symbols) environments. Data are expressed as mean $\pm$ SE of nine sheep.

than in the thermoneutral environment (Fig. 2 and Table 3). Blood glucose concentrations changed ($P < 0.05$) initially from the preinfusion values, and were maintained at the preinfusion levels during the latter period of the hyperinsulinemic euglycemic clamp experiment. The glucose infusion rate continued to increase initially, but it was almost stable during the last hour of the 2-hour period in both environments. The GIR was higher ($P < 0.01$) during cold exposure than in the thermoneutral environment.

Discussion

Environmental temperatures above or below the thermoneutral zone will increase energy needs²⁾. KENNEDY and MILLIGAN¹⁾ showed that DM intake increased and DM digestibility decreased during cold exposure (2–5°C) compared with a thermoneutral environment (22–25°C) in sheep. However, DM intake of sheep was not influenced by cold exposure (0°C) in the present experiment. This discrepancy might be involved in species and forms of diets used. Perhaps, the sheep consumed the diet at maximum even in the thermoneutral

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Table 3. The concentrations of blood glucose and glucose infusion rate in the hyperinsulinemic euglycemic clamp experiment in sheep given a roughage diet *ad libitum*

	Thermoneutral		Cold exposure		Significance
	Mean	SE	Mean	SE	
Basal glucose (mg/dl)	50	1	56	2	*
Clamped glucose (mg/dl)	49	1	55	3	NS
GIR ^a (mg/kg/min)	3.16	0.22	5.80	0.41	**

^aGIR = glucose infusion rate during the last 40 min of glucose infusion period.

* $P < 0.05$

** $P < 0.01$

NS = not significant

environment. It has been reported that insulin responsiveness to glucose was decreased and tissue responsiveness to insulin was enhanced during cold exposure (0°C) in sheep using the glucose clamp technique^{3,11}. These experiments were carried out in sheep fed fixed levels of diets. Insulin responsiveness to glucose was enhanced and tissue responsiveness to insulin tended to be enhanced in sheep received a high energy diet⁸, and heat production in animals consuming fibrous diets was higher¹⁰. Therefore, it is possible that the responses to cold exposure of insulin responsiveness to glucose and tissue responsiveness to insulin in sheep given *ad libitum* access to roughage might differ from those obtained in sheep fed fixed levels of diets^{3,11}. However, in the present experiment, the effect of cold exposure on insulin responsiveness to glucose and tissue responsiveness to insulin was similar in sheep fed fixed levels of diets^{3,11}. A possible reason for this might be unchanged DM and estimated metabolizable energy intakes of sheep during cold exposure.

In the hyperglycemic clamp experiment the GIR was higher ($P < 0.05$) during cold exposure than in the thermoneutral environment. This may be due to the accelerated glucose turnover rate during cold exposure⁹. Although the MPII during glucose infusion was not different, the ratio of MPII to GIR (insulin responsiveness to glucose) was lower ($P < 0.05$)

during cold exposure than in the thermoneutral environment. This suggested that insulin responsiveness to glucose was decreased during cold exposure. The reduced insulin responsiveness to glucose during cold exposure might be mediated via activation of adrenergic α -receptor of sympathetic nervous system as described by SASAKI and TAKAHASHI^{6,7}. The similar results were obtained in sheep fed a high-concentrate diet, but the ratio was unchanged during cold exposure in sheep fed a high-roughage diet using the hyperglycemic clamp technique³.

Although plasma insulin concentrations were not determined in the hyperinsulinemic euglycemic clamp experiment, plateaus in plasma insulin concentrations were reported in sheep infused at the same rate as the present experiment (6 mU/kg/min for 2 hours)⁴. Both blood glucose concentrations and the glucose infusion rate were stable during the last hour of insulin infusion in both environments. Therefore, the GIR (tissue responsiveness to insulin) was comparable, and were higher ($P < 0.01$) during cold exposure than in the thermoneutral environment. Dose-response experiments of hyperinsulinemic euglycemic clamp were not carried out, however, WEEKES *et al.*¹¹ suggested that cold exposure might increase the responsiveness of postreceptor events to insulin action without affecting insulin binding to its receptor. Therefore, tissue responsiveness to

insulin during cold exposure was enhanced due to increases in intracellular disposal of glucose accompanied by depletion of glycogen in the insulin-sensitive tissues such as the portal drained viscera and skeletal muscle. In conclusion, insulin responsiveness to glucose was reduced and tissue responsiveness to insulin was enhanced during cold exposure in sheep given a roughage diet *ad libitum* without changing in voluntary intakes.

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粗飼料自由摂取めん羊におけるグルコースに対する
インスリン分泌能および組織に対するインスリン
作用に及ぼす寒冷暴露の影響

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粗飼料を自由摂取させためん羊においてグルコースに対するインスリン分泌能および組織に対するインスリン作用に及ぼす寒冷暴露の影響を測定するために, HGC (hyperglycemic clamp) 法およびEGC (hyperinsulinemic euglycemic clamp) 法が適用された. 乾物摂取量は寒冷暴露によって影響されなかった. HGC 法ではグルコース注入速度に対する血漿インスリン濃度増加の割合 (グルコースに対するインスリン分泌能) は寒冷暴露によって低下した ($P<0.05$). EGC 法ではグルコース注入速度 (組織に対するインスリン作用) は常温時よりも寒冷暴露時に高かった ($P<0.01$). 以上の結果から, 粗飼料を自由に摂取させても寒冷暴露によって採食量が増加しなかっためん羊では寒冷暴露時にグルコースに対するインスリン分泌能は低下し, 組織に対するインスリン作用は増加することが示唆された.

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