INSULIN RESPONSIVENESS TO GLUCOSE AND TISSUE RESPONSIVENESS TO INSULIN IN COWS, SHEEP AND PIGS

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Summary

Insulin responsiveness to glucose and tissue responsiveness to insulin, using the hyperglycemic clamp and the hyperinsulinemic euglycemic clamp techniques, were compared among cows, sheep and pigs. The plasma insulin concentrations during the hyperglycemic clamp period were highest ($p < 0.05$) in cows, followed by sheep and pigs. The glucose infusion rate in the hyperinsulinemic euglycemic clamp technique was greater ($p < 0.01$) in pigs than in cows and sheep. These results suggest that insulin responsiveness to glucose is higher in cows and lower in pigs, and suggest that tissue responsiveness to insulin is higher in pigs than in cows and sheep.
(Key Words: Insulin, Glucose Clamp, Cow, Sheep, Pig).

Introduction

Insulin plays a central role for nutrient metabolism in nonruminants and ruminants. Insulin secretory response is considerably different between them. Insulin secretory response to feeding is almost same in both animals (Ambo et al., 1973). This is considered to be due to the increased blood glucose concentration in nonruminant animals, but in ruminants blood glucose concentration changed little after feeding (Bassett, 1972; Sasaki et al., 1984). Moreover, volatile fatty acids stimulate insulin secretion in ruminants, but do not in nonruminant animals (Manns and Boda, 1967; Horino et al., 1968). Both hepatic glucose production and peripheral glucose utilization to insulin were less sensitive in sheep than in human (Weekes et al., 1983).

The present experiment was designed to compare insulin responsiveness and tissue responsiveness to insulin using the glucose clamp technique (DeFronzo, 1979) among cows, sheep and pigs.

Materials and Methods

Animals

Four Japanese Shorthorn cows, eight Suffolk rams and five crossbred (Hampshure x Landrace) pigs were used in the present experiment.

The cows, aged 2 years and weighing about 650 kg, were housed in stalls. They were daily fed 4 kg of mixed concentrate (71% TDN, 20.9% crude protein) and to provide ad libitum orchardgrass hay (50% TDN, 7.3% crude protein) at 0900 and 1700 h. Two polyethylene catheters were inserted into both jugular veins before the experiment.

The rams, aged 1 to 2 years and weighing about 50 kg, were used. They were surgically prepared with a skin loop enclosing the left carotid artery. The animals were kept in metabolic cages in a laboratory room, and were fed 2% body weight (BW) of lucerne hay cubes (49% TDN, 17.8% crude protein) and 0.5% BW of a commercial concentrate (67% TDN, 18.9% crude protein) once daily at 1700 h. A polyethylene catheter for infusions was inserted into a femoral vein at least 2 days before the experiment. A catheter for blood sampling was placed into the carotid artery loop at 2 hours before the experiment. The pigs, aged 3 months and weighing about 30 kg, were kept in metabolic cages in a laboratory room. They were fed 2% BW of the commercial concentrate diet (82% TDN, 18.5% crude protein) twice daily at 0900 and 1700 h. Two silicone rubber catheters were inserted into both jugular veins under anathesia at least 2 weeks before the experiment as described by Takahashi (1986b).

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Received July 27, 1990
Accepted November 17, 1990

The catheters were filled with 3.8% sterile solution of trisodium citate.

Experimental Procedures

Two glucose clamp techniques, the hyperglycemic clamp technique and the hyperinsulinemic euglycemic clamp technique, were carried out in the present experiment.

In the hyperglycemic clamp technique the desired blood glucose concentration was designed to be the basal glucose concentration (a mean of three determinations immediately before infusion) plus 50 mg/100 ml, and the sterile solution of glucose (34.8% for cows and 20.0% for sheep and pigs) was infused by a multichannel peristaltic pump (Model AC-2120, Atto Co. Ltd., Japan) through the jugular catheter for 2 h. Blood samples were taken from the other catheter at 5-min intervals, and blood glucose concentration was determined within 1 min after each blood sampling. Then the glucose infusion rate was adjusted to the blood glucose concentration being the desired goal. The blood sampling for insulin assay and the monitor of glucose infusion rate were performed 10-min intervals throughout the 2-h infusion period.

After the determination of the preinfusion blood glucose in the hyperinsulinemic euglycemic clamp technique, porcine insulin (Actrapid monocomponent porcine insulin: Novo Industry, Denmark) dissolved in 0.9% sodium chloride and 2.5% potassium chloride was infused by the peristaltic pump through the jugular catheter at the constant rate of 6 $mU \cdot kg^{-1} \cdot min^{-1}$ as described previously (Sano et al., 1990). The glucose solution was also infused by the other peristaltic pump through the same catheter to blood glucose concentration being the preinfusion levels. The monitors of blood glucose concentration and the glucose infusion rate and the blood sampling for insulin assay were performed during the 2-h period as described in the hyperglycemic clamp technique.

Analyses

After blood glucose concentration was determined by an automated glucose analyzer (Model GLU-1, Toa Electronics Ltd., Japan), blood samples were centrifuged in the cold (10,000 x g, 10 min, 4℃). Plasma insulin was assayed by a radioimmunoassay kit (IRI 'Eiken', Eiken Chemical Co. Ltd., Japan) which used porcine insulin for standard and anti insulin guinea pig serum for antiserum. Bovine and ovine insulin (Sigma Chem. Co., U.S.A.) was used for insulin assay of cows and sheep, respectively.

Calculations

The steady state glucose infusion rate (SSGIR) was defined as the mean value calculated during 60 to 120 min of the experimental period (Weekes et al., 1983). The mean plasma insulin increment (MPII) was also calculated during 60 to 120 min of infusion (Sano et al., 1990). Insulin responsiveness to glucose and tissue responsiveness to insulin were represented by the MPII in the hyperglycemic clamp technique and the SSGIR in the hyperinsulinemic euglycemic clamp technique, respectvely.

Data are expressed as mean ± SD. Differences were evaluated by Student's paired *t*-test to compare means in the preinfusion period with values obtained after the initiation of infusion. Differences among species were analyzed by one way ANOVA. When *F*-test was significant ($p < 0.05$), Tukey's multiple range test was used for statistical analysis.

Results and Discussion

Basal States

All blood samples were obtained through the catheters without stress. The highest ($p < 0.05$) basal blood glucose concentrations were observed in pigs, followed by cows and sheep (table 1). The basal insulin concentrations were higher ($p < 0.01$) in cows than in the other species. Both basal blood glucose and plasma insulin concentrations in these species were comparable to the data reported previously (Sartin et al., 1985; Takahashi, 1986a; Sano et al., 1990).

Glucose Clamp

In the hyperglycemic clamp technique, blood glucose concentrations in all species were increased by the exogenous glucose infusion, reached a desired goal within 1 h and were almost clamped at the desired goal thereafter (figure 1), though the blood glucose concentrations in pigs were considerably fluctuant compared with those in cows and sheep during the latter half of the 2h period. The actual concentrations of blood

TABLE 1. BASAL BLOOD GLUCOSE AND PLASMA INSULIN CONCENTRATIONS IN COWS, SHEEP AND PIGS

	Cow	Sheep	Pig
Blood glucose (mg/100 ml)	52 ± 1^{b}	45 ± 2^{c}	72 ± 5^{a}
Plasma insulin (μU/ml)	33 ± 2^{A}	14 ± 6^{B}	15 ± 3^{B}

Means in a same row without common superscript letters differ (^{A,B} $p < 0.01$; ^{a,b,c} $p < 0.05$)

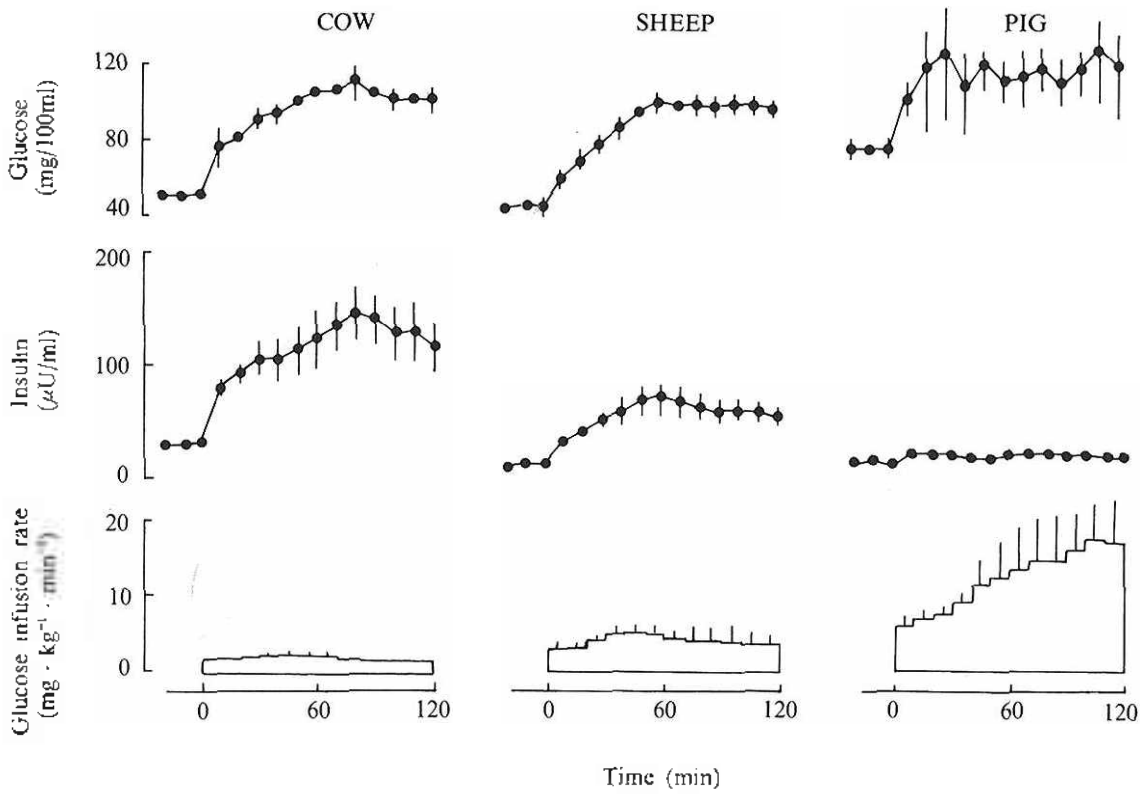

Figure 1. Blood glucose and plasma insulin concentrations and glucose infusion rate before and during the hyperglycemic clamp technique in cows, sheep and pigs.

glucose were $103 \pm 1\%$, $101 \pm 1\%$ and $92 \pm 2\%$ of the desired goal during the latter half of the 2-h period in cows, sheep and pigs, respectively. The glucose infusion rate in all species increased progressively as the infusion time proceeded during the first half of the 2-h period. In cows and sheep, the glucose infusion rate was relatively stable during the latter half of the 2-h period, but in pigs it continued to increase. The SSGIR in pigs was higher ($p < 0.01$) than those in cows and sheep (table 2). Plasma insulin concentrations responded to hyperglycemia in all animals ($p < 0.01$), and the greatest increment of plasma insulin was observed in cows, followed by sheep and pigs. The MPII were higher ($p < 0.05$) in cows and lower ($p < 0.05$) in pigs than in the other two species. Though the dosages of glucose were different, the insulin secretory response to the intravenous injection of glucose was considerably lower in pigs (Takahashi, 1986a; Gopinath and Etherton, 1989) than in sheep (Sasaki and Takahashi, 1983) and cows (Sartin et al., 1985; Denbow et al., 1986) on the base of the insulin secretory response to infused glucose ratio.

In the hyperinsulinemic euglycemic clamp

TABLE 2. GLUCOSE INFUSION RATE AND PLASMA INSULIN CONCENTRATION DURING THE HYPERGLYCEMIC CLAMP TECHNIQUE IN COWS, SHEEP AND PIGS

	Cow	Sheep	Pig
SSGIR[a] (mg · kg^{-1} · min^{-1})	1.8 ± 0.4[c]	4.1 ± 2.0[c]	15.6 ± 4.9[a]
MPII[a] (μU/ml)	102 ± 48[b]	48 ± 21[c]	6 ± 3[d]

[a]See text

Means in a same row *without common superscript* letters differ ([a,c] $p < 0.01$; [b,c,d] $p < 0.05$)

technique, blood glucose concentrations in cows and sheep were almost clamped at the preinfusion levels during the 2-h period of insulin infusion by the simultaneous glucose infusion and were 99 ± 3% of the desired goal during the latter half of 2-h period in both species (figure 2). Blood glucose concentrations in pigs initially decreased, then recovered to the initial levels and were 101 ± 16% of the desired goal during the latter half of the 2 h period. The glucose infusion rate initially increased progressively, and was stable during the latter half of the 2-h period in all species. The SSGIR in pigs was higher ($p < 0.01$) than those in sheep and cows (table 3). The high tissue responsiveness to insulin in pigs should not be due to the porcine insulin used, since the SSGIR/MPII ratio in the hyperglycemic clamp technique, which is one of the indicators of insulin sensitivity (DeFronzo, 1979), was also higher ($p < 0.01$) in pigs than in cows and sheep. Moreover, our findings showed that insulin sensitivity of peripheral tissues in pigs was comparable with those in human (Rizza et al., 1981) and rats (Farrell et al., 1988).

The present results confirmed that there were great differences in insulin responsiveness and

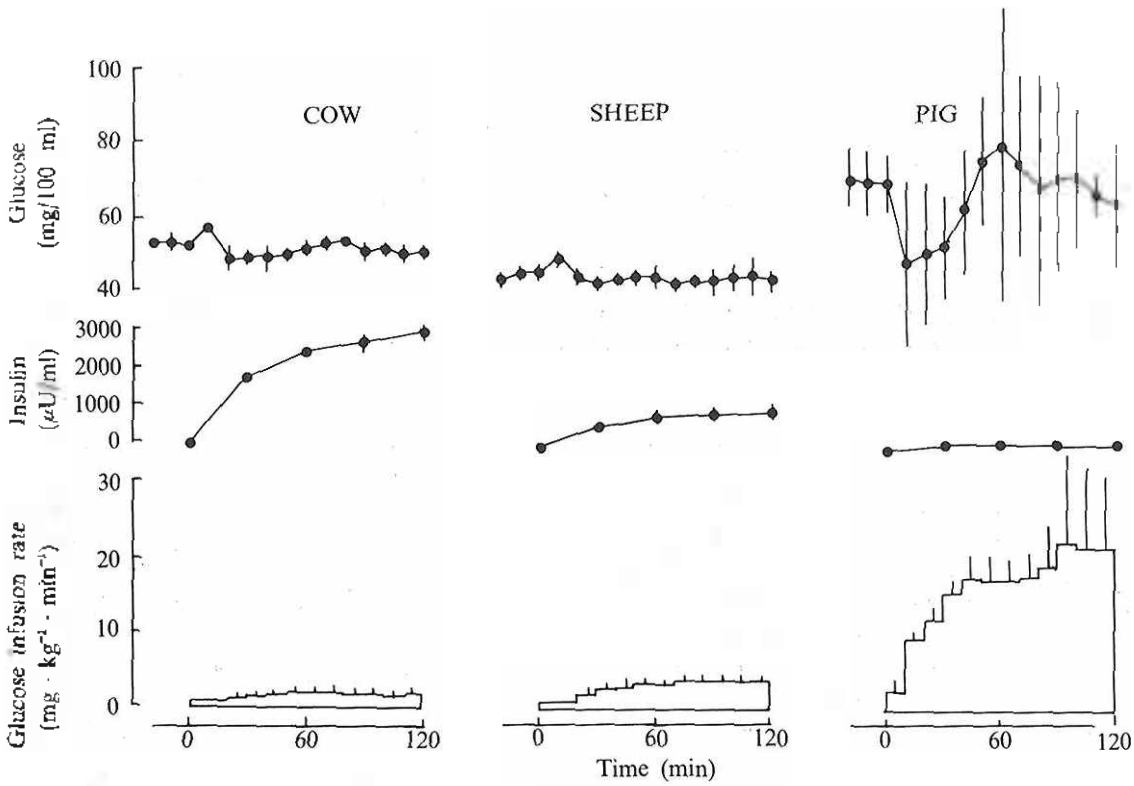

Figure 2. Blood glucose and plasma insulin concentrations and glucose infusion rate before and during the hyperinsulinemic euglycemic clamp technique in cows, sheep and pigs.

TABLE 3. GLUCOSE INFUSION RATE AND PLASMA INSULIN CONCENTRATION DURING THE HYPER INSULINEMIC EUGLYCEMIC CLAMP TECHNIQUE IN COWS, SHEEP AND PIGS

	Cow	Sheep	Pig
SSGIR[a] ($mg \cdot kg^{-1} \cdot min^{-1}$)	2.1 ± 0.4^{C}	4.1 ± 0.9^{C}	20.9 ± 7.0^{B}
MPII[a] ($\mu U/ml$)	2660 ± 149^{B}	879 ± 184^{C}	186 ± 81^{D}

[a]See text

Means in a same row without common superscript letters differ (^{B,C,D} $P < 0.01$)

sensitivity between nonruminant and ruminant animals, though the mechanism could not be explained. The MPII in the hyperinsulinemic euglycemic clamp technique was extremely low ($p < 0.01$) in pigs (table 3). This suggests that a large quantity of insulin secreted from the pancreas is taken by the target tissues and may be a reason for the high insulin sensitivity in pigs.

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