INTRODUCTION

The effect of insulin on nutrient metabolism is determined by insulin concentration in body fluid and insulin action to the body tissue. Insulin action is distinguished between tissue responsiveness to insulin and insulin sensitivity (Kahn, 1978). In addition to physiological effect, such as lactation, cold exposure and obesity (Weekes et al., 1991), the nutritional effects on tissue responsiveness to insulin or insulin sensitivity have been reported previously. Sano and Terashima (2001) showed the modification of insulin action by crude protein (CP) intake in cold exposed sheep. Sano and Fujita (2006) reported that calcium propionate supplementation tended to enhance tissue responsiveness to insulin in sheep. Furthermore, we reported that increases in metabolizable energy (ME) intake as starch (Fujita et al., 2000) and intake of energy substrates other than CP (Fujita et al., 2006b) might enhance tissue responsiveness to insulin and insulin sensitivity, respectively, in goats.

Although these previous researches showed the modification of insulin action by levels of intake of some nutrients, comparisons between effects of various nutrients on insulin action are little conducted in ruminants. Regarding carbohydrates, Obara et al. (1991) described that a readily fermentable carbohydrate, which includes cereal starch (ST), sugar and sugar-based extract, improved energy and nitrogen metabolism in ruminants, but the extent of its effects depended on its type and form, feeding levels of both the carbohydrate and the basal diet, and type of basal diet. Indeed, Chamberlain et al. (1993) reported that sucrose (SU), lactose, xylose and fructose, unlike ST, produced a decrease in acetate proportion and increases in propionate or butyrate proportions with increased microbial protein synthesis in the rumen. This suggests the possibility that the
effects on insulin action may be different between dietary carbohydrates.

The present study compared the effects of dietary ST, SU and their mixture (ST+SU) on tissue responsiveness to insulin and insulin sensitivity in goats using a hyperinsulinemic euglycemic clamp technique with four doses of insulin infusion. Changes in concentrations of plasma metabolites and insulin relating to feeding were also examined to evaluate the effects of the carbohydrates on metabolic state in the body.

MATERIALS AND METHODS

Animals and management

Four Saanen intact male goats (2 to 4 years, initial mean body weight (BW) 39.6±3.8 kg standard error of means (SEM)) were used. To collect blood samples, two goats were surgically prepared under anesthesia with a skin loop enclosing the left carotid artery. The other two were not prepared with the loop and thus blood samples were taken from the left jugular vein during the study. Each animal was kept individually in a metabolic cage in a controlled environment chamber at air temperature of 20±1°C and offered the diets of 1.2 times of ME and CP for maintenance requirements (National Research Council, 1985). Seventy percent of the ME intake was given as alfalfa hay (ME 1.82 Mcal/kg, CP 14.7%), ground corn (ME 2.78 Mcal/kg, CP 7.5%) and ground soybean meal (ME 2.77 Mcal/kg, CP 46.3%) at a weight ratio of 1, 1 and 0.3, respectively. Residual ME intake was provided through ST (ME 3.07 Mcal/kg, CP 0%), ST+SU (weight ratio of 1.0 and 0.91 for ST and SU, respectively; ME 3.20 Mcal/kg, CP 0%) or SU (ME 3.35 Mcal/kg, CP 0%) (Agriculture, Forestry and Fisheries Research Council Secretariat, MAFF, 1995). Thus, the percentage of alfalfa hay in the whole diet was 33%. The period of each dietary treatment was 21 d. The animals were paired one with and the other without the skin loop to minimize the effect of sampling sites on the data obtained. The one pair was allotted to an ascending order of the amount of sucrose (ST, ST+SU and then SU diets), and the other pair to a descending order (SU, ST+SU and then ST diets). The allotment of treatment allowed all animals to be subjected to all three treatments. The diets were equally divided into two meals, one offered at 8:30 and the other at 20:30 h. Animals had free access to water and mineral blocks, and completely ate the diets within 15 min after feeding. Surgery, management, and blood sampling were carried out according to the guidelines established by the Animal Care Committee of Iwate University.

Blood samplings with the time relating to feeding

A catheter for blood sampling was inserted into the left jugular vein of two goats without the skin loop on 16th d of each dietary treatment, and into the left carotid artery of other two goats with skin loop 1 h after feeding of the morning meal on 17th d of each dietary treatment. These catheters were filled with a sterile solution of trisodium citrate (38 g/L). Three blood samples (10 ml each) were taken via the catheters at 10-min intervals during 2.5 to 3 h after feeding on 17th d of each dietary treatment. These samples were taken as background samples in an isotope dilution experiment (Fujita et al., 2007). The arterial catheter was removed immediately after the end of the isotope dilution experiment, but the jugular catheter was kept by filling it with the sterile solution of trisodium citrate with regular flushing until the hyperinsulinemic euglycemic clamp experiment was ended on 21st d of each dietary treatment.

Additionally, on 18th, 19th and 20th d of each dietary treatment, blood sampling (10 ml) was done via the jugular catheter or by carotid artery puncture with the skin loop 6 h after feeding once a day.

All blood samples were placed in heparinized tubes and stored in crushed ice immediately after sampling. Plasma was separated from blood by centrifuging at 8,000×g for 10 min at 4°C and stored at -20°C until analysis of plasma metabolites and insulin.

The concentrations of plasma metabolites and insulin at 3 h after feeding were obtained as mean values of the three samples taken during 2.5 to 3 h after feeding, and those at 6 h after feeding were taken as averages of three values on 18th, 19th and 20th d. In addition, the concentrations at 13 h after feeding were obtained by averaging three values of samples taken during 30 min before insulin infusion in the hyperinsulinemic euglycemic clamp experiment as described below. The concentrations at 3, 6 and 13 h after feeding, although taken on different days, were obtained under the same feeding conditions, and hence were combined to evaluate the variation relating to feeding and the modification of the variation with different carbohydrates.

Hyperinsulinemic euglycemic clamp experiment

On 21st d of each dietary treatment, the hyperinsulinemic euglycemic clamp experiment was conducted over 8 h beginning at 10:00 h to evaluate tissue responsiveness to insulin and insulin sensitivity (Bergman et al., 1985). A catheter for infusion was inserted into the right jugular vein of all goats on 5 d before the experiment, and another for blood sampling was inserted into the left carotid artery of two goats with the skin loop at least 2 h before the initiation of the experiment. In the other two goats without the skin loop, the left jugular catheter, which was kept as described above, was used for blood sampling.
Analysis

Glucose concentrations in blood and plasma were measured with the automated glucose analyzer described above. Plasma lactate concentration was measured as described by Taylor (1996). Plasma urea nitrogen concentration was determined by a modified diacetylmonoxim method (Coulombe and Favreau, 1963). Plasma free fatty acids (FFA) concentration was enzymatically determined using a diagnostic kit (NEFA-C test wako, Wako, Osaka, Japan). Plasma total amino nitrogen concentration was determined by a dinitrophenol method (Rapp, 1963). Plasma insulin concentration was measured using a radioimmunoassay kit (IRI 'Eiken', Eiken Chemical Co. Ltd., Tokyo, Japan). Plasma concentrations of individual volatile fatty acids (VFA) were measured as described by Sano et al. (1989) with slight modifications. Plasma (2 ml) was deproteinized by adding 1 ml of sodium tungstate solution (100 g/L) and 1 ml of 0.34 M H₂SO₄, and the whole supernatants was distilled with steam. The distillates fixed with 10 mM NaOH were dried at 90°C, resolved with 1.7 M H₃PO₄ and then applied to gas chromatography (HP5890A, Hewlett Packard, Avondale, USA).

Calculations

In the hyperinsulinemic euglycemic clamp experiment, the mean concentrations of blood glucose and plasma insulin, and the mean glucose infusion rate (GIR) were calculated during the last hour of each 2-h period of insulin infusion. The GIR is considered to indicate the overall effects of insulin on whole body glucose metabolism, because insulin stimulates peripheral glucose utilization and suppresses hepatic glucose production (Bergman et al., 1985). Tissue responsiveness to insulin and insulin sensitivity were evaluated on the basis of the maximal GIR (GIRₘₐₓ) and the plasma insulin concentration at half-maximal GIR (EC₅₀, Bergman et al., 1985). These indices were calculated from logistic regression of individual dose-response curves for GIR vs. ln (insulin concentration) before insulin infusion and during the last hour of 0.38, 0.96, 2.4 and 6.0 mU/kg BW/min of insulin infusion (Clement et al., 1996).

Statistical analysis

Data for blood samples obtained from different sites were combined in data analysis, because the sampling from the same site in the same animal in any treatment allows to compare the effects of type of carbohydrate even if there are differences in absolute values between sampling sites. All data (n = 4) were analyzed using the MIXED procedure of SAS (SAS Institute Inc., 1996). The data for the concentrations of plasma metabolites and insulin in relation to feeding were analyzed by a split-plot design with repeated measures for the time relating to feeding. A main-plot was a group (ascending or descending order of SU intake), a sub-plot was the type of carbohydrate, and sub-sub-plots were the time relating to feeding and an interaction between type of carbohydrate and the time. Autoregressive order one was used as a covariance structure for repeated measures. In the hyperinsulinemic euglycemic clamp experiment, the mean concentrations of blood glucose and plasma insulin, and the GIR were analyzed by a split-plot design with repeated measures for insulin infusion levels, with a group as a main-plot, type of carbohydrate as a sub-plot, insulin infusion levels and type of carbohydrate×insulin infusion levels interaction as sub-sub-plots, and autoregressive order one as a covariance structure for repeated measures. The GIRₘₐₓ and EC₅₀ were also

All catheters were flushed and filled with a sterile solution of trisodium citrate (38 g/L). Animals were not given the morning meal on the day of the experiment. Blood samples (10 ml each) were taken at 10-min intervals over 30 min before the initiation of insulin infusion (corresponding to 13 h after feeding), and their glucose concentrations were immediately determined with an automated glucose analyzer (GLU-1, Erma Optical Works, Tokyo, Japan). Residual blood samples were placed in heparinized tubes and stored in crushed ice until centrifugation for assay of plasma metabolites and insulin. Basal glucose concentration was obtained by averaging these glucose concentrations. Thereafter, sterile saline solutions (9 g NaCl/L) of insulin (Novolin R, Novo Nordisk, Denmark) were continuously infused during four sequential 2-h periods at rates of 0.38, 0.96, 2.4 and 6.0 mU/kg BW/min, respectively, via the infusion catheter using a peristaltic pump (AC-2120, Atto Co. Ltd., Tokyo, Japan). The concentrations of insulin solution were 64 U/L of sterile saline for the two lower infusion doses and 400 U/L of sterile saline for the two higher infusion doses. A sterile solution of glucose (200 g/L) was infused with another peristaltic pump at variable rates through the same infusion catheter, to maintain basal glucose concentration. During the insulin infusion, 1 ml of blood was taken from the catheters for blood sampling at 10-min intervals, with additional 1 ml taken at 30-min intervals. Blood glucose concentration was immediately determined to maintain preinfusion glucose levels by changing glucose infusion rates as required. Residual blood samples taken at 30-min intervals were placed in heparinized tubes and stored in crushed ice until centrifugation. The glucose infusion rates were recorded every 10 min during the infusion. After the end of the clamp experiment, plasma was separated from blood by centrifuging at 8,000×g for 10 min at 4°C and stored at -20°C until use for metabolites or insulin assay.

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analyzed by a split-plot design with a group as a main-plot and type of carbohydrate as a sub-plot. In each analysis of variance, significances for the main effects and interactions were taken as the p<0.05 level and tendencies as the 0.05≤p<0.10 level. Comparisons among each level within the main effects were conducted by the least squares difference with Tukey-Kramer adjustment at p<0.10. No effect of order of carbohydrate treatment was considered in the present study, because the group effect was not significant (p>0.05) in any data.

RESULTS

Changes in plasma metabolites and insulin in relation to feeding

The effects of type of carbohydrate on the changes in concentrations of plasma metabolites and insulin in relation to time after feeding are shown in Figure 1. Plasma glucose concentration was higher (p = 0.01) for SU diet than for ST and ST+SU diets, and low (p<0.01) at 13 h after feeding compared with 3 and 6 h after feeding. Plasma lactate concentration was not affected by the type of carbohydrate (p = 0.22), but had a higher value (p<0.01) at 3 h after feeding than at 6 and 13 h after feeding. There was a significant interaction between the type of carbohydrate and the time relating to feeding (p = 0.01) for lactate concentration; it was higher for SU diet compared with ST and ST+SU diets at 3 h after feeding, although differences between dietary treatments were not observed at 6 and 13 h after feeding. Plasma FFA concentration was similar (p = 0.98) among dietary treatments, but was higher (p<0.01) at 13 h after feeding than at 3 and 6 h after feeding. Plasma urea nitrogen concentration showed a lack of the effect of type of carbohydrate (p = 0.49), but had a lower value...
For urea nitrogen, although an interaction between the type of carbohydrate and the time relating to feeding was not significant (p = 0.19), its value was lower for SU diet than for ST and ST+SU diets at 3 h after feeding and similar for each dietary treatment at 6 and 13 h after feeding. Plasma total amino nitrogen concentration showed a non-significant low value (p = 0.10) for ST diet compared with ST+SU and SU diets. The concentration linearly increased (p<0.01) with time relating to feeding. Plasma insulin concentration was unaffected (p = 0.44) by the type of carbohydrate but showed a linear decrease (p<0.01) with time relating to feeding. Increasing SU intake linearly decreased (p<0.01) plasma acetate concentration. Although not significantly affected, plasma propionate and n-butyrate concentrations were increased (p = 0.17) and decreased (p = 0.17) in values, respectively, with increasing SU intake. All of these VFA concentrations were linearly decreased (p<0.01) with time relating to feeding and an interaction between the type of carbohydrate and the time relating to feeding was not significant (p≥0.10); the value for plasma propionate was higher for SU diet than for ST and ST+SU diets at 3 h after feeding, but was similar for each dietary treatment at 6 and 13 h after feeding (p = 0.14).

Hyperinsulinemic euglycemic clamp

Blood glucose concentration was maintained at very close to the basal values by the variable rate of glucose infusion in all experiments (Table 1). Plasma insulin concentration and glucose infusion rate were relatively constant during the 2nd h of each 2-h period of insulin infusion (data not shown) and increased dose-dependently (p<0.01) with increasing rate of insulin infusion. The mean plasma insulin concentration (p = 0.93) and GIR (p = 0.62) during insulin infusion were unaffected by the type of carbohydrate. There were no interactions between the type of carbohydrate and insulin in these measurements (p≥0.10). The response curves of GIR to increasing plasma insulin concentration in each dietary treatment are shown in Figure 2; the shapes of the curves were visually similar for all dietary treatments. The GIR_{max} (p = 0.54) and EC_{50} (p = 0.54) were not significantly different between dietary treatments (Table 1).

DISCUSSION

It is cautioned that the results obtained in the present study should be carefully interpreted because of the limited number of animals used. The increased plasma glucose concentration at 6 h after feeding than at 3 and 13 h after feeding. For urea nitrogen, although an interaction between the type of carbohydrate and the time relating to feeding was not significant (p = 0.19), its value was lower for SU diet than for ST and ST+SU diets at 3 h after feeding and similar for each dietary treatment at 6 and 13 h after feeding. Plasma total amino nitrogen concentration showed a non-significant low value (p = 0.10) for ST diet compared with ST+SU and SU diets. The concentration linearly increased (p<0.01) with time relating to feeding. Plasma insulin concentration was unaffected (p = 0.44) by the type of carbohydrate but showed a linear decrease (p<0.01) with time relating to feeding. Increasing SU intake linearly decreased (p<0.01) plasma acetate concentration. Although not significantly affected, plasma propionate and n-butyrate concentrations were increased (p = 0.17) and decreased (p = 0.17) in values, respectively, with increasing SU intake. All of these VFA concentrations were linearly decreased (p<0.01) with time relating to feeding and an interaction between the type of carbohydrate and the time relating to feeding was not significant (p≥0.10); the value for plasma propionate was higher for SU diet than for ST and ST+SU diets at 3 h after feeding, but was similar for each dietary treatment at 6 and 13 h after feeding (p = 0.14).
concentration with increasing SU intake across the time (related to feeding) is similar to the previous results in sheep that have shown a trend for an increase in plasma glucose concentration by SU supplementation (Obara and Dellow, 1993; Obara et al., 1994). The decreased plasma acetate concentration with SU across the time may be attributed to a decrease in ruminal acetate production, because SU decreased ruminal acetate concentration and proportion without changing ruminal total VFA concentration at 6 h after feeding (Fujita et al., 2007). In ruminants, blood acetate mostly originates from acetate produced in the digestive tract, mainly the rumen (Pethick et al., 1981), and acetate is metabolized less by the portal-drained viscera and the liver than propionate and butyrate (Bergman, 1990). In contrast, although other plasma metabolites and insulin concentrations were not affected by SU intake across the time, a significant interaction on lactate and weak interactions on urea nitrogen and propionate (greater differences between ST and SU diets for these concentrations at 3 h after feeding than at 6 and 13 after feeding) were detected. Similar tendencies in values with a lack of statistical significance were found on other metabolites and insulin concentrations, except for FFA and total amino nitrogen. These results suggest that SU may greatly affect nutrient metabolism during the early period after feeding, rather than through the postfeeding period. The strong effects of SU during the early period after feeding could be explained by the rapidity of fermentation of SU as reported by Chamberlain et al. (1985), who have found more rapid and greater decline in ruminal pH with SU than with ST in sheep. The present results for plasma metabolites and insulin concentrations suggest that SU may not be greatly different from ST on effects of nutrients metabolism because of its transient effects, when these carbohydrates are fed twice daily.

We expected that responses of tissue responsiveness and sensitivity to insulin would be different between SU and ST feeding, because previous reports had shown an improvement in dietary nitrogen utilization and an enhancement in glucose metabolism by SU supplementation (Obara et al., 1994; Sutoh et al., 1996). However, no effects of different carbohydrates on GIRmax or EC50 in the present study suggest that SU may be similar to ST on the effects on the tissue responsiveness and sensitivity to insulin. The plasma insulin concentrations during the clamp experiments are considered to be physiologically significant levels at two lower doses of insulin infusion (Bassett, 1974; Sutton et al., 1988; Metcalf and Weekes 1990), but to be very high levels at two higher doses. Thus, the values of EC50 in the present study are also likely to be in the physiological range of plasma insulin concentration. No differences between ST and SU in responses of tissue responsiveness or sensitivity to insulin may be associated with the lack of persistency of SU effects on nutrients metabolism in the body as described above. Fujita et al. (2006b) have suggested that a possible enhancement in insulin sensitivity with increasing intake of energy substrates other than CP was accompanied by decreased plasma urea nitrogen and FFA and increased plasma total amino nitrogen through 3, 6 and 13 h after feeding, and by increased plasma propionate at 3 and 6 h after feeding. An increase in feeding frequency may be effective for an improvement in persistency of SU effect. Indeed, the strong effects of SU supplementation on energy and nitrogen metabolism have been found in sheep fed 12 or 24 times daily (Obara and Dellow, 1993; Obara et al., 1994; Sutoh et al., 1996).

A response to a readily fermentable carbohydrate feeding in ruminants depends on the level of feeding of the carbohydrate and the type of basal diet, as well as type and form of the carbohydrate (Obara et al., 1991). In the present study, the diet used was a high-concentrate diet with 33% of alfalfa hay. In contrast, previous researches that used alfalfa hay alone have demonstrated the greater effects of SU supplementation on energy and nitrogen metabolism (Obara and Dellow, 1993; Obara et al., 1994; Sutoh et al., 1996). When ST and some sugars were supplemented to sheep fed grass silage alone, Chamberlain et al. (1985, 1993) also found the difference between ST and SU in pH, VFA pattern and ammonia nitrogen concentration in the rumen, and microbial nitrogen supply to the small intestine. These results suggest that a ratio of roughage to concentrate in a
diet may modify the effects of SU on nutrients metabolism, and also that use of a high-concentrate diet as an experimental diet may be a cause of the little response to SU in plasma metabolites and insulin concentrations and tissue responsiveness and sensitivity to insulin in the present study. This may be supported by a trend for enhancement in tissue responsiveness to insulin by calcium propionate supplementation in sheep fed a diet containing 80% lucerne hay in other study (Sano and Fujita, 2006).

The major effects of SU on energy and nitrogen metabolism in the previous studies may involve the effects of increasing energy intake beside the effects of SU itself, because energy intake was higher by about 30% for the sucrose supplemented diet compared with the control diet (Obara and Dellow, 1993; Obara et al., 1994; Sutoh et al., 1996). We have reported that increasing energy intake with starch would enhance nitrogen retention and whole body protein synthesis (Fujita et al., 2006a) and tissue responsiveness to insulin (Fujita et al., 2000), and that increasing energy intake attributed to ingredients other than CP may be associated with enhanced insulin sensitivity (Fujita et al., 2006b).

In conclusion, as far as the effects on the concentrations of plasma metabolites and insulin, and tissue responsiveness and sensitivity to insulin in adult goats are concerned, SU may not be greatly different from ST when the carbohydrates are supplemented to a high-concentrate diet and fed twice daily. However, the possibility cannot be excluded that the responses to SU in these variables may be modified by feeding frequency, the composition of diets and energy intake.

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