Species Difference in Plasma Insulin Responses to Intravenous Xylitol in Man and Several Mammals

TAKESHI KUZUYA*, YASUNORI KANAZAWA*, MITSUAKI HAYASHI**,
MASATOSHI KIKUCHI* AND TAKEHIKO IDE*

*Third Department of Internal Medicine, Faculty of Medicine,
University of Tokyo, Hongo, Tokyo
and

**Hokkaido Branch of National Institute of Animal Health,
Hitsuji-ga-oka, Sapporo

Synopsis

Glucose and xylitol were administered intravenously to man and several mammals in the same doses, and the plasma insulin responses were compared. Marked species differences were found. In man, 12 g or 30 g of glucose produced definitely greater rises of plasma insulin than xylitol. In cows and goats, both 0.2 g/kg glucose and xylitol increased plasma insulin markedly and to a similar extent. Plasma insulin responses of horses to 0.2 g/kg glucose and xylitol were rather sluggish and poor. In rabbits, the peak insulin levels following 0.2 g/kg glucose and xylitol were approximately similar or slightly higher after glucose, but with the dose of 1.0 g/kg, the peak plasma insulin value after xylitol was a little higher than after glucose. In rats, glucose produced higher insulin peaks than xylitol both at the doses of 0.2 g/kg and 1.0 g/kg. These results are in contrast to the results obtained in dogs, in which xylitol has a definitely stronger insulin-releasing effect than glucose. The cause for this species variation is not explained at present.

Xylitol has a stronger stimulatory effect on insulin secretion than glucose in dogs (Kuzuya *et al.*, 1966; 1969; Hirata *et al.*, 1966; 1968). Its insulin-releasing effect was also demonstrated in the isolated rat islet *in vitro* (Montague and Taylor, 1968). In man, however, a relatively small or no increase in plasma insulin concentration was observed following the intravenous administration of xylitol (Kuzuya *et al.*, 1967; Geser *et al.*, 1967). Recently, Wilson and Martin (1970) demonstrated that the insulinotropic effect of xylitol was less marked than that of glucose in rhesus monkeys. In view of the discrepancy in insulin responses in these previous results, we investigated the insulinotropic effect of xylitol in several different mammal species. Equivalent doses of glucose and xylitol were given intravenously to cows, goats, horses, rabbits, rats, dogs and also to healthy human volun-

teers. The results revealed that the relative plasma insulin responses to xylitol and glucose differed markedly among different species.

Methods

Human experiments

Volunteers consisted of healthy male students and doctors, 21–37 years of age, weighing 55–73 kg, with no history of diabetes or metabolic diseases. None of them were obese. The experiments were performed in the morning after overnight fast. They had reclined supine for about half an hour before the first blood sampling. Indwelling needles filled with 10% solution of sodium citrate were inserted into both arm veins. After taking two control samples at 10–15 min intervals through one of these needles, 12 g or 30 g (40 ml of 30% or 60 ml of 50% solution) of glucose or xylitol was administered through another needle over 3 min (for 12 g) or 10 min (for 30 g). 10–12 blood samples were withdrawn for 90 min after the injection.

Animal experiments

Cows, goats, horses and rabbits were subjected to

the experiments without anesthesia after an overnight fast. The weight and sex of these animals are shown in each figure. In cows, goats and horses, blood was taken through a Teflon indwelling canula inserted into the jugular vein. In rabbits, blood was collected directly into glass tubes from a cut on the ear vein. About 10 blood samples, each 2–4 ml, were collected before and after the administration of glucose or xylitol to each species. Wistar-strain rats weighing 300–500 g were used after fasting for 4–5 hr. After anesthesia with the intraperitoneal injection of about 30 mg/kg sodium pentobarbital, a thin polyethylene catheter, filled with dilute heparin in saline, was inserted into the jugular or femoral vein for blood sampling. About 30 min later, glucose or xylitol solution was injected into the jugular vein and 3–5 samples (0.8–1.0 ml each) were collected before and after the injection from each animal.

Glucose and xylitol were given intravenously in a dose of 0.2 g/kg or 1.0 g/kg in 10–50% solution. Except for rabbits and rats, the same individual animals were tested twice at an interval of 1–2 weeks with the equivalent doses of both glucose and xylitol. The order of glucose and xylitol experiments was randomized. Cows and horses were given the injection by infusion into the jugular vein within 10 min. The injection took 2–3 min for goats, and less than one minute for rabbits and rats. The end of injection was

designated as zero time for all species.

Both in man and animal experiments, blood samples were collected into glass tubes containing heparin powder, and centrifuged within one hour. The plasma was kept at -20°C for subsequent analysis.

Measurements of glucose, xylitol and insulin

After deproteinization with $\text{Ba}(\text{OH})_2\text{-ZnSO}_4$ (Somogyi, 1945), glucose was determined by a glucose-oxidase method and xylitol by a periodate oxidation method as described elsewhere (Kuzuya *et al.*, 1969). Plasma insulin was measured by a double-antibody radioimmunoassay (Kanazawa *et al.*, 1966), with the standard insulin of each animal species, except for the goat samples which were assayed with a pork insulin standard. Anti-pork insulin serum was used for all assays.

Prior to the immunoassay, the optimal condition for immunoprecipitation by the second precipitating rabbit antiserum was carefully selected to insure maximum precipitation in the presence of plasma of each animal species.

Results

Intravenous administration of 12 g or 30 g

Fig. 1. Changes in plasma glucose, xylitol and insulin concentrations (mean $\pm$ SE) following intravenous administration of 12 g glucose or xylitol in 7 male volunteers.

Fig. 2. Changes in plasma glucose, xylitol and insulin concentrations (mean $\pm$ SE) following intravenous administration of 30 g glucose or xylitol in 7 male volunteers.

of glucose or xylitol to man (Figs. 1 and 2)

The effects of the same doses of glucose and xylitol were compared in the same subjects, but the experiments with 12 g and 30 g were conducted in different groups. These doses corresponded approximately to 0.2 and 0.5 g/kg.

Infusion of glucose increased plasma glucose and insulin immediately. The peak insulin levels were 31–288 μ U/ml after 12 g and 55–230 μ U/ml after 30 g glucose. The plasma glucose returned to the fasting level in 30 min after 12 g glucose and in 45 min after 30 g glucose. The return of plasma insulin was also slightly delayed following a higher dose.

Following xylitol administration, plasma glucose did not change or slightly increased. Plasma xylitol reached 79–129 and 171–295 mg/100 ml after 12 g and 30 g xylitol respectively, and disappeared mostly in 60–90 min.

Plasma insulin increased in all cases but to a very variable extent. The peaks were 15–113 μ U/ml after 12 g xylitol and 15–46 μ U/ml after 30 g xylitol. The mean plasma insulin curves were similar after administration of 12 g and 30 g xylitol.

Intravenous administration of 0.2 g/kg glucose or xylitol to cows (Fig. 3.)

Plasma glucose concentration increased from the fasting level of 55–82 mg/100 ml to 220–249 mg/100 ml immediately after administration of glucose and decreased to the control level in 90–120 min. Plasma insulin concentration also increased promptly from the fasting level of 30–59 μ U/ml to the peak of 65–480 μ U/ml in 10 min, and returned to the fasting level within 120 min.

After intravenous injection of xylitol, plasma glucose increased slightly in 3 cows,

Fig. 3. Changes in plasma glucose and insulin concentrations following intravenous administration of 0.2 g/kg glucose or xylitol in 4 cows. Different symbols represent different individuals.

and plasma insulin rose to the peak of 95–680 $\mu\text{U}/\text{ml}$ in 10 min, then decreased to the fasting level in 90–120 min. Cow no. 1 which showed the maximal insulin response to glucose was also the best responder to xylitol administration, and cow no. 2 responded least to both glucose and xylitol. Maximal insulin levels following xylitol injection were higher than those after glucose in 3 animals.

Intravenous administration of 0.2 g/kg glucose or xylitol to goats (Fig. 4.)

After intravenous administration of glucose, plasma glucose increased from the fasting value of 59–68 mg/100 ml to 264–283 mg/100 ml, then decreased gradually. Plasma insulin

level was 52–97 $\mu\text{U}/\text{ml}$ at fasting. It reached the peak of 111–291 $\mu\text{U}/\text{ml}$ at 10 min after injection, and returned to the fasting level in 60–90 min.

Following intravenous administration of xylitol, plasma glucose rose slightly. Plasma insulin increased markedly, reaching the maximum of 114–385 $\mu\text{U}/\text{ml}$ in 10–30 min. In 3 animals the maximal plasma insulin levels after xylitol injection were higher than those after glucose. Plasma xylitol reached 116–329 mg/100 ml immediately after injection, then decreased promptly.

Intravenous administration of 0.2 g/kg glucose or xylitol to horses (Fig. 5.)

Fig. 4. Changes in plasma glucose, xylitol (dotted line) and insulin concentrations following intravenous administration of 0.2 g/kg glucose or xylitol in 4 goats. Different symbols represent different individuals.

Plasma glucose increased from the fasting level of 82–94 mg/100 ml to 222–243 mg/100 ml after intravenous injection of glucose, and then gradually decreased, but remained higher than the fasting level even at 120 min. The increase in plasma insulin after glucose injection was rather sluggish, and it was not clear in horse no. 3.

Intravenous administration of xylitol caused slight or moderate rises of plasma glucose. Plasma insulin increased slightly and slowly in horses no. 1 and no. 2, but did not rise in horse no. 3. Plasma xylitol reached a peak of 129–136 mg/100 ml immediately after injection, and disappeared from the circula-

tion within 60 min.

Intravenous administration of 0.2 or 1.0 g/kg of glucose or xylitol to rabbits (Figs. 6 and 7)

These unanesthetized rabbits had fairly high and variable fasting plasma glucose levels. Following injection of 0.2 g/kg glucose, plasma glucose reached 250–330 mg/100 ml, and returned to the control level in 60 min. Injection of 0.2 g/kg xylitol caused slight or moderate rises in plasma glucose. Plasma xylitol reached 131–255 mg/100 ml immediately after xylitol injection and almost disappeared in 60 min. Plasma insulin reached

Fig. 5. Changes in plasma glucose, xylitol and insulin concentrations following intravenous administration of 0.2 g/kg glucose or xylitol in 3 horses. Different symbols represent different individuals.

the peak of 13–57 $\mu\text{U/ml}$ at 0 or 10 min after administration of 0.2 g/kg glucose, and of 11–44 $\mu\text{U/ml}$ at 10 minutes after 0.2 g/kg xylitol.

Following intravenous administration of 1.0 g/kg glucose, plasma glucose increased to 529–607 mg/100 ml and plasma insulin reached a peak of 19–118 $\mu\text{U/ml}$ in 0–5 min. Intravenous administration of 1.0 g/kg xylitol caused variable rises in both plasma glucose and plasma insulin. Plasma insulin reached a peak of 42–151 $\mu\text{U/ml}$ between 30–45 min after injection, which was delayed in comparison with the peak after the injection of glucose. Plasma xylitol rose to 347–398 mg/100 ml following injection, and declined thereafter approaching the baseline in 120 min.

Intravenous administration of 0.2 or 1.0 g/kg of glucose or xylitol to rats (Figs.

8 and 9)

Plasma glucose ranged from 111 to 146 mg/100 ml before injection of glucose or xylitol. It rose to 169–241 or 435–568 mg/100 ml following 0.2 or 1.0 g/kg glucose, and returned to the control level in 30 or 60 minutes. When glucose was given, plasma insulin increased from 13–56 $\mu\text{U/ml}$ to 31–192 $\mu\text{U/ml}$ after 0.2 g/kg and to 35–264 $\mu\text{U/ml}$ after 1.0 g/kg within 5 min, then decreased. Following administration of xylitol, plasma glucose tended to increase slightly or remain unchanged, and plasma xylitol reached 43–70 mg/100 ml after 0.2 g/kg and 303–431 mg/100 ml after 1.0 g/kg, respectively. The peak insulin concentrations were 45–103 $\mu\text{U/ml}$ after 0.2 g/kg xylitol and 54–108 $\mu\text{U/ml}$ after 1.0 g/kg xylitol. The peak of insulin response curve was slightly delayed after a higher dose of xylitol but not after a higher dose of glucose.

Fig. 6. Changes in plasma glucose, xylitol and insulin concentrations following intravenous administration of 0.2 g/kg glucose or xylitol in 5 rabbits. Different symbols represent different individuals.

Discussion

Although the secretory response of insulin to various stimuli has been investigated in a variety of conditions both *in vivo* and *in vitro*, only a few reports have dealt with the differences in insulin secretion among different animal species. However, some previous studies did suggest that the insulinotropic effects of some substances are not uniform in various animal species. For example, intravenous galactose increases plasma insulin in sheep (Boda, 1964), but not in man (Karam *et al.*, 1966). Some short chain fatty acids are reported to stimulate insulin secretion strongly in sheep and cows, but have no effect in rats, rabbits and pigs (Horino *et al.*, 1968). In the

present study, we compared the relative insulinotropic potencies of intravenous glucose and xylitol in several mammals including man. We gave these two substances on the same weight dose basis. As xylitol has a smaller molecular weight, these doses are not equimolar, but the number of carbon atoms per the same weight are approximately the same.

Glucose is usually considered as a common and most important physiological factor controlling insulin secretion in mammals, and its insulinotropic effect has been confirmed in many species. An increase in plasma insulin after a glucose load was observed in all the species tested in this study, but the degree of hyperinsulinemia from the same dosage per body weight was markedly different between different species. The insulinotropic effects of

Fig. 7. Changes in plasma glucose, xylitol (dotted line) and insulin concentrations following intravenous administration of 1.0 g/kg glucose or xylitol in 5-6 rabbits. Different symbols represent different individuals.

xylitol were also observed in all the animals except horses in which the rise of plasma insulin was not significant. These data suggest that insulin-releasing effect of xylitol is fairly widely distributed in mammals. However, species variability in the effects of xylitol appears much greater than that of glucose. Among animals studied so far, dogs are the most responsive animal to xylitol administration. Man is a poor responder to xylitol, and horses responded weakly both to xylitol and glucose (Fig. 10). Rhesus monkeys also appear to be poor responders to xylitol (Wilson and Martin, 1970).

Our data show that the responsiveness to these stimuli is variable even in the same species. Within the same species, there ap-

peared to be a general tendency that an individual which responded well to glucose with a marked rise to plasma insulin responded well also to xylitol. Such individual variation of plasma insulin responses to xylitol was also observed in the experiments on man.

As glucose and xylitol were given to cows, horses and goats only in a dose of 0.2 g/kg, the relative insulin-releasing potencies of glucose and xylitol in a larger dose is unknown. In rabbits, glucose produced a little larger effect than xylitol at 0.2 g/kg, while xylitol caused a greater hyperinsulinemia than glucose when 1.0 g/kg was given. The dose-response relationships of the insulin-releasing effect of xylitol are particularly remarkable in dogs (Kuzuya *et al.*, 1969). At 0.05-0.1 g/kg,

Fig. 8. Changes in plasma glucose, xylitol (dotted line) and insulin concentrations following intravenous administration of 0.2 g/kg glucose or xylitol in anesthetized rats. Each value represents the mean $\pm$ SE of 5-9 experiments.

glucose and xylitol produce approximately similar increases in plasma insulin, while with doses more than 0.2 g/kg, xylitol produced much greater effect than glucose. Such dose-dependency was not so clear in rats or in man. However, it seems possible that the insulinemic effect of xylitol in some other animals might be more pronounced if it is given in larger doses.

Although a direct effect on the pancreas was suggested both for glucose and xylitol *in vivo* (Kuzuya and Kanazawa, 1969) and *in vitro* (Montague and Taylor, 1968), there was some difference between the patterns of insulin responses following large doses of glucose and xylitol. In most animals, plasma insulin reached a peak within 10 minutes after injection of glucose, irrespective of dose, whereas the maximal plasma insulin level following 1.0 g/kg xylitol appeared at 15-30 min or later in rats, rabbits and dogs. This

fact may suggest that xylitol has some additional effect *in vivo* for promoting insulin secretion.

One of the current concepts for the mechanism of insulin secretion by sugars is that sugars must be metabolized within the islet cells to stimulate insulin release (Coore and Randle, 1964; Montague and Taylor, 1969). Consequently, it is possible that species differences of plasma insulin responses to glucose and xylitol are caused by the species differences in the metabolism of these two substances in the islet cells. Ruminant animals which utilize short-chain fatty acids as a primary source for body energy, respond to short-chain fatty acids with a greater rise of plasma insulin than after glucose (Horino *et al.*, 1968). However, it is also possible that the physiological effect of overnight fast may differ between ruminant and non-ruminant,

Fig. 9. Changes in plasma glucose, xylitol (dotted line) and insulin concentrations following intravenous administration of 1.0 g/kg glucose or xylitol in anesthetized rats. Each value represents the mean $\pm$ SE or 5-9 experiments.

or between big or small-sized animals. Thus, the difference in basal conditions of various animal species may have contributed to the observed difference in insulin response to some extent. However, the ability of xylitol to release insulin seems to be unrelated to the size of animals, whether or not they are ruminant, and whether they are herbivorous or carnivorous. Our few preliminary experiments suggest that insulin secretion by xylitol is not marked in cats. Lack of information about the metabolic characteristics of islet cells of each species precludes a precise explanation of our results. However, these data indicate that precautions are needed, in the

investigation of insulin secretion, to extend the conclusion obtained in one animal, to other animal species.

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Fig. 10. Comparison of mean plasma insulin curves following intravenous administration of 0.2 g/kg or 1.0 g/kg of glucose (left) or xylitol (right) in man and several mammals.

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