

Long-term sucrose-drinking causes increased body weight and glucose intolerance in normal male rats

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The current epidemic of diabetes likely reflects marked changes in environmental factors, although genetic susceptibility plays a powerful role in the occurrence of diabetes in certain populations. We investigated whether long-term sucrose-drinking causes hyperglycaemia in male Wistar-Imamichi littermates (n 32), which are not genetically susceptible to diabetes or obesity. Each litter was divided equivalently into two groups, the sucrose group and the control group. The sucrose group received 300 g/l sucrose water and the control group received regular water until 42 weeks of age. Rats were weighed every 1 or 2 weeks. Oral glucose tolerance tests were performed at 28 and 36 weeks of age. Plasma glucose and insulin concentrations were measured. Body weights were significantly greater in the sucrose group than in the control group in 18-week-old rats ($P < 0.05$), and the difference between the two groups reached 163 g by the end of the study ($P < 0.01$). The 120 min post-load plasma glucose concentration in the sucrose group was 11.4 (SD 2.8) mmol/l in 28-week-old rats and 12.7 (SD 2.2) mmol/l in 36-week-old rats, while that of the control group remained approximately 7.3–7.7 mmol/l. In the sucrose group, the plasma insulin peak occurred 30 min post-load at 28 weeks of age; but the peak disappeared and hyperinsulinaemia was prolonged at 36 weeks of age. In conclusion, long-term sucrose-drinking causes increased body weight and glucose intolerance in normal male rats.

Dietary sucrose: Experimental diabetes mellitus: Type 2 diabetes mellitus: Environmental: Litter size: Wistar rats

The increased prevalence of diabetes in the USA has continued unabated through the late 1990s to the present day (Mokdad *et al.* 2001). The prevalence of diabetes in Japan and other developed countries is also believed to be increasing. Although genetic susceptibility plays a powerful role in the occurrence of diabetes in certain populations, population gene pools shift quite slowly. Therefore, the current epidemic likely reflects marked changes in environmental factors, especially lifestyle. Lifestyle changes, characterized by decreased physical activity and increased energy consumption, have promoted obesity, which is a remarkably strong risk factor for diabetes (Edelstein *et al.* 1997). A striking increase in the prevalence of obesity in the USA has been reported (Kuczmarski *et al.* 1994). Other developed countries are most likely experiencing a similar increase in obesity prevalence. In regard to dietary components, fat intake appears to be an important determinant of diabetes risk, independent of total caloric intake (Tsunehara *et al.* 1990; Marshall *et al.* 1997). On the other hand, metabolic studies of the risks and benefits of sugar ingestion have been controversial. Janket *et al.* (2003) reported that sugar intake does not play a deleterious role in primary prevention of type 2 diabetes. However, a recent prospective study from Ludwig *et al.* (2001) showed a clear-cut, grade relation between the consumption of sugar-sweetened drinks and the development of obesity in children. Moreover, James *et al.* (2004) reported that a school-based education programme produced a modest reduction in the number of carbonated

drinks consumed, which was associated with a reduction in the number of overweight and obese children. In the present study, we investigated whether environmental factors cause hyperglycaemia in an ordinary strain of rats that is not genetically susceptible to diabetes or obesity. We studied littermates divided equally into two groups, as we considered them genetically closer. One group received 300 g/l sucrose water and the other group received regular water until 42 weeks of age. We compared glucose tolerance and body weight between the two groups.

Experimental methods

Experimental animals

Wistar-Imamichi rats, which were established by Dr Tomonori Imamichi in 1960, are the offspring of Wistar standard strain lot no. 1859, of which three breeding pairs were brought to Japan in 1938. Wistar-Imamichi rats are an ordinary experimental strain used in Japan. They do not develop obesity and diabetes under normal conditions. All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Six breeding pairs of Wistar-Imamichi rats were purchased from the Institute for Animal Reproduction (Ibaragi, Japan). The rats were bred in the laboratory animal unit of our university and produced thirty-two male offspring. The litters remained with their parents until 3 weeks of age. After matching

Abbreviations: FPG, fasting plasma glucose; IVGTT, intravenous glucose tolerance test; OGTT, oral glucose tolerance test; PG, plasma glucose.

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the body weights in each of the litters, each litter was divided equally into two groups, a sucrose group and a control group. The rats were housed individually in an environmentally controlled room with a 12 h light–dark cycle (light from 7.00 to 19.00 hours). Rats had free access to food and a designated type of water (sucrose water or regular water). Rats in the sucrose group (n 16) received 300 g/l sucrose water and rats in the control group (n 16) received regular water from 3 to 42 weeks of age. All animals received the same basal non-purified diet (MF, Oriental Yeast Co. Ltd, Tokyo, Japan) (Table 1).

Measurement of body weight

Rats were weighed every week from 3 to 16 weeks of age and every 2 weeks from 16 to 42 weeks of age. Weight was measured by an A & D electronic balance (A & D Co. Ltd, Tokyo, Japan) at 9.00 hours.

Fasting blood samples and intravenous and oral glucose tolerance tests

Intravenous glucose tolerance tests (IVGTTs) were performed on all rats at 8 and 16 weeks of age. Oral glucose tolerance tests (OGTTs) were performed on all rats at 28 and 36 weeks of age. Fasting blood samples only were obtained from each rat at 12 and 24 weeks of age. Food was removed and 300 g/l sucrose water was changed to regular water at 18.00 hours 1 d before the tests. At 9.00 hours a fasting blood sample (1000 μ l) was taken from a peripheral subcutaneous vein of the neck. Rat hairs had been shaved and skin had been revealed on the region at 1 d before the blood collections. We were able to detect a peripheral subcutaneous vein of the region and punctured it with a fine needle (external diameter 0.45 mm, length 13.0 mm) fitted with a 1.0 ml volume syringe. Then, glucose (1 g/kg) was administered using a 500 g/l glucose solution (Otsuka, Tokushima, Japan) that was loaded via a peripheral subcutaneous vein of the inguinal region or oral catheter. Rat hairs had been shaved and skin had been revealed on the region at 1 d before the IVGTTs. We were able to detect a peripheral subcutaneous vein of the region and punctured it with a fine needle (external diameter 0.45 mm, length 13.0 mm) fitted with a 2.5 ml volume syringe which was filled with a 500 g/l glucose solution. Blood samples were taken alternately from the peripheral subcutaneous veins on each side of

the neck at 30 min (200 μ l) and 120 min (1000 μ l) after loading for the IVGTT in 8-week-old rats. Serial blood samples (1000 μ l) were taken alternately from the peripheral subcutaneous veins on each side of the neck at 30, 60 and 120 min after loading for the IVGTT in 16-week-old rats, as well as for the OGTTs in 28- and 36-week-old rats. All procedures were performed without anaesthesia by an investigator who was experienced in the handling of rats. The rats were restrained for a short amount of time during oral catheterization and blood collection. Pre-implanted cannulae were not used in the present study.

Measurements of plasma glucose and insulin concentrations

After blood samples were obtained, part of the sample was used to immediately measure plasma glucose (PG) by Novo Assist Plus (Novo Nordisk, Denmark). Each sample was measured twice. The remaining part of the blood sample was immediately centrifuged. The plasma was obtained and stored at -40°C until assayed. Plasma insulin concentrations, which were measured by Mercodia Rat Insulin ELISA systems (Mercodia, Uppsala, Sweden), were examined at fasting and 120 min after loading for the IVGTT in 8-week-old rats and at fasting, 30 min, 60 min and 120 min after loading for the IVGTT in 16-week-old rats and the OGTTs in 28- and 36-week-old rats. Plasma insulin concentrations were measured in six representative rats per group. Each sample was measured in duplicate.

Statistical analyses

Data are presented as means and their standard deviations. For normally distributed data, statistical analysis was performed with a Student's paired or unpaired t test. For any difference in distribution between groups, Wilcoxon's signed-rank test or Welch's unpaired t test was performed. All analyses were done with Stat View software package version 4.5 (Abacus Concepts, Berkeley, CA, USA). P values <0.05 were considered statistically significant.

Results

Comparison of body weights between the control and sucrose groups

As shown in Fig. 1 and Table 2, rat body weights were significantly greater in the control group (n 16) than in the sucrose group (n 16) during the younger period between 4 and 9 weeks of age (the small inserted graph). Then, rat body weights were significantly greater in the sucrose group than in the control group at 18 weeks of age (control group, 486 (SD 44) g; sucrose group, 517 (SD 45) g; $P<0.05$). The difference in body weights between the two groups increased as the rats grew. At the end of the study, the difference in mean body weights between the two groups was 163 g (control group, 636 (SD 74) g; sucrose group, 799 (SD 100) g; $P<0.01$), i.e. the sucrose group became 25.6% heavier than the control group.

Serial changes in fasting plasma glucose and insulin concentrations

Fasting plasma insulin concentrations in the sucrose group and the control group were equivalent at 8 weeks of age. Then, the fasting

Table 1. Composition of the commercial diet*

Component	Amount (g/100 g diet)
Water	7.0
Protein	24.0
Lipid	5.1
Fibre	2.7
Ash	3.2
Nitrogen-free extracts	54.5
Mineral mixture†	3.2
Vitamin and amino acid mixture‡	0.18

MF, Oriental Yeast, Tokyo, Japan.

† The mineral mixture provided (g/kg diet): calcium, 11.0; phosphorus, 8.3; magnesium, 2.5; sodium, 2.6; potassium, 7.5; iron, 0.16; zinc, 0.49; copper, 0.074; manganese, 0.49.

‡ The vitamin and amino acid mixture provided (per kg diet): retinol, 315 mg; cholecalciferol, 308 μ g; α -tocopherol, 63 mg; menadione, 0.4 mg; thiamine, 19 mg; riboflavin, 13 mg; pyridoxine HCl, 8 mg; niacin, 40 mg; pantothenic acid, 19 mg; biotin, 0.2 mg; inositol, 600 mg; choline, 1 g; folic acid, 0.4 mg; ascorbic acid, 54 mg.

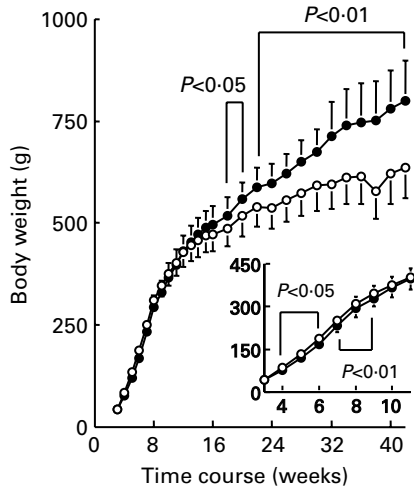


Fig. 1. Comparison of body weight between the control group and the sucrose group. As shown in the small inserted graph, rat body weights were significantly greater in the control group (○, *n* 16) than in the sucrose group (●, *n* 16) during the younger period between 4 and 9 weeks of age. Then, rat body weights were significantly greater in the sucrose group than in the control group at 18 weeks of age (*P*<0.05). The difference in body weights between the two groups increased as the rats grew. Values are means with their standard deviations depicted by vertical bars.

plasma insulin concentration in the sucrose group increased to six-fold that in the control group at 12 weeks of age (*P*<0.01), although no difference in body weights was observed between the two groups (Table 2). After 12 weeks of age, the fasting plasma insulin concentration in the sucrose group remained at least three times higher than that in the control group. Fasting plasma glucose (FPG) concentrations in the sucrose group were significantly higher than those in the control group at 28 (*P*<0.01) and 36 (*P*<0.05) weeks of age. No significant difference in FPG was observed at 8, 12 and 24 weeks of age.

Intravenous glucose tolerance tests at 8 weeks of age

PG concentrations in the sucrose group (*n* 16) were significantly (*P*<0.05) higher than those in the control group (*n* 16) at 30 min (control group, 11.2 (SD 2.5) mmol/l; sucrose group, 14.3 (SD 2.1) mmol/l) and 60 min (control group, 9.2 (SD 2.1) mmol/l; sucrose

group, 12.3 (SD 2.1) mmol/l) after loading (Fig. 2), although no significant difference between the two groups was observed in FPG (Table 2). No significant difference between the two groups was observed in plasma insulin concentrations at fasting and 120 min after loading (control group (*n* 6), 0.31 (SD 0.23) µg/l; sucrose group (*n* 6), 0.46 (SD 0.31) µg/l).

Intravenous glucose tolerance tests at 16 weeks of age

PG concentrations in the sucrose group (*n* 16) were significantly higher than those in the control group (*n* 16) at 30 min (control group, 10.8 (SD 2.5) mmol/l; sucrose group, 14.5 (SD 2.1) mmol/l), 60 min (control group, 8.9 (SD 1.3) mmol/l; sucrose group, 12.5 (SD 2.7) mmol/l) and 120 min (control group, 6.7 (SD 1.0) mmol/l; sucrose group, 8.3 (SD 2.1) mmol/l) after loading (*P*<0.01; Fig. 3(A)), while FPG levels were significantly lower in the sucrose group than in the control group (*P*<0.05; Table 2). Plasma insulin concentrations in the sucrose group (*n* 6) were significantly higher than those in the control group (*n* 6) at all time-points (control group, 0.95 (SD 0.56) µg/l at 30 min, 1.05 (SD 0.70) µg/l at 60 min, 0.86 (SD 0.52) µg/l at 120 min; sucrose group, 3.53 (SD 0.51) µg/l at 30 min, 3.64 (SD 0.74) µg/l at 60 min, 2.94 (SD 0.91) µg/l at 120 min; *P*<0.01; Fig. 3(B)).

Oral glucose tolerance tests at 28 weeks of age

PG concentrations in the sucrose group were significantly higher than those in the control group at all time-points (control group, 8.1 (SD 1.5) mmol/l at 30 min, 10.0 (SD 2.5) mmol/l at 60 min; sucrose group, 11.0 (SD 1.3) mmol/l at 30 min, 13.6 (SD 1.9) mmol/l at 60 min; *P*<0.01; Fig. 4(A)). PG concentrations remained 11.4 (SD 2.8) mmol/l at 120 min after loading in the sucrose group, while they remained 7.3 (SD 0.9) mmol/l in the control group. Plasma insulin concentrations in the sucrose group were significantly greater than those in the control group at fasting (*P*<0.01), 30 min (control group, 1.13 (SD 0.55) µg/l; sucrose group, 3.42 (SD 0.55) µg/l; *P*<0.01), 60 min (control group, 1.45 (SD 0.36) µg/l; sucrose group, 2.85 (SD 0.82) µg/l; *P*<0.05) and 120 min (control group, 1.08 (SD 0.31) µg/l; sucrose group, 2.85 (SD 0.74) µg/l; *P*<0.01) after loading. In the sucrose group, the peak in plasma insulin concentrations occurred 30 min after loading (Fig. 4(B)).

Table 2. Serial changes of fasting plasma glucose (FPG) and insulin over 36 weeks followed in control and sucrose-loading groups.

	Weeks											
	8		12		16		24		28		36	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control (<i>n</i> 16)												
FPG (mmol/l)	4.45	0.43	4.48	0.38	4.87	0.59	4.91	0.71	5.21	0.58	5.00	0.48
Insulin (µg/l)‡	0.12	0.08	0.13	0.09	0.62	0.49	0.57	0.35	0.64	0.35	0.56	0.31
Weight (g)	310	23	428	35	471	40	537	50	573	56	614	67
Sucrose loading (<i>n</i> 16)												
FPG (mmol/l)	4.43	0.58	4.54	0.57	4.49	0.48†	5.24	0.98	5.88	0.61*	5.61	0.27†
Insulin (µg/l)‡	0.13	0.10	0.82	0.48*	1.84	0.58*	2.08	1.01*	1.90	0.82*	2.78	1.73†
Weight (g)	293	30†	430	39	497	45	597	49*	650	53*	748	94*

**P*<0.01, †*P*<0.05 mean values were significantly different from the control group, ‡Insulin measured in six representative cases in both groups.

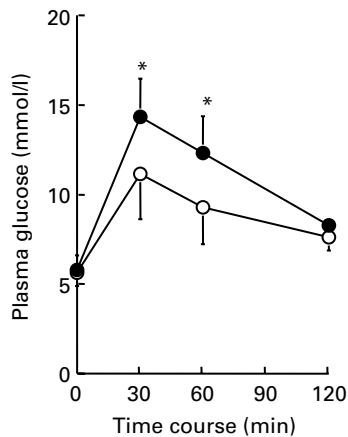


Fig. 2. Intravenous glucose tolerance test at 8 weeks of age. Glucose (1 g/kg) was loaded via a peripheral subcutaneous vein of the inguinal region. Plasma glucose concentrations in the sucrose group (●, *n* 16) were significantly ($*P < 0.05$) higher than those in the control group (○, *n* 16) at 30 and 60 min after loading, although there was no significant difference in fasting plasma glucose between the two groups. Values are means with their standard deviations depicted by vertical bars.

Oral glucose tolerance tests at 36 weeks of age

PG concentrations in the sucrose group were significantly higher than those in the control group at fasting ($P < 0.05$), 30 min (control group, 10.1 (SD 3.3) mmol/l; sucrose group, 14.7 (SD 2.2) mmol/l; $P < 0.01$), 60 min (control group, 10.4 (SD 3.6) mmol/l; sucrose group, 15.2 (SD 2.2) mmol/l; $P < 0.05$) and 120 min (control group, 7.7 (SD 2.2) mmol/l; sucrose group, 12.7 (SD 2.2) mmol/l; $P < 0.01$) after loading (Fig. 5(A)). PG concentrations at 120 min after loading in the sucrose group were higher than at 28 weeks of age, while they remained at approximately 7.7 mmol/l in the control group. Plasma insulin concentrations in the sucrose group were significantly higher than those in the control group at fasting ($P < 0.05$), 30 min (control group, 1.27 (SD 0.41) μ g/l; sucrose group, 3.93 (SD 1.95) μ g/l; $P < 0.05$), 60 min (control group, 1.50 (SD 0.25) μ g/l; sucrose group, 4.49 (SD 1.42) μ g/l; $P < 0.01$) and 120 min (control group, 1.16 (SD 0.12) μ g/l; sucrose group, 4.29 (SD 1.02) μ g/l;

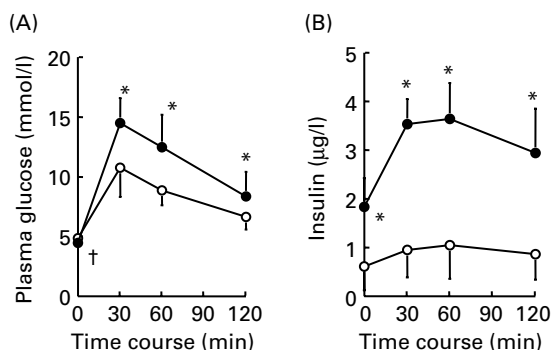


Fig. 3. Intravenous glucose tolerance test at 16 weeks of age. Glucose (1 g/kg) was loaded via a peripheral subcutaneous vein of the inguinal region. (A), Plasma glucose concentrations in the sucrose group (●, *n* 16) were significantly ($*P < 0.01$) higher than those in the control group (○, *n* 16) at 30, 60 and 120 min. Fasting plasma glucose concentrations were significantly lower in the sucrose group than in the control group ($\dagger P < 0.05$). (B), Plasma insulin concentrations in the sucrose group (●, *n* 6) were significantly higher than those in the control group (○, *n* 6) at all time-points ($*P < 0.01$). Values are means with their standard deviations depicted by vertical bars.

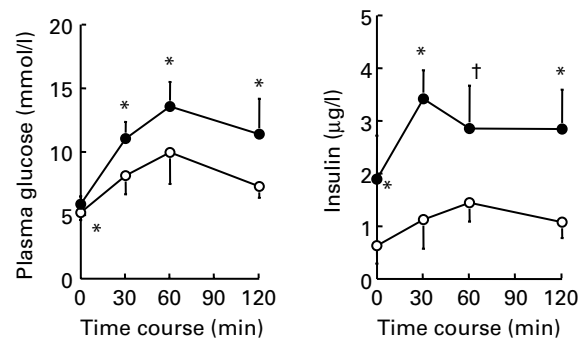


Fig. 4. Oral glucose tolerance test at 28 weeks of age. Glucose (1 g/kg) was loaded via oral catheter. (A), Plasma glucose concentrations in the sucrose group (●, *n* 16) were significantly higher than those in the control group (○, *n* 16) at all time-points ($*P < 0.01$). (B), Plasma insulin concentrations in the sucrose group (●, *n* 6) were significantly greater than those in the control group (○, *n* 6) at fasting ($*P < 0.01$), 30 min ($*P < 0.01$), 60 min ($\dagger P < 0.05$) and 120 min ($*P < 0.01$) after loading. Values are means with their standard deviations depicted by vertical bars.

$P < 0.01$) after loading. In the sucrose group, the plasma insulin peak at 30 min after loading that was observed at 28 weeks of age had disappeared and hyperinsulinaemia was prolonged (Fig. 5(B)).

Discussion

The present study demonstrated that long-term sucrose-drinking causes increased body weight and glucose intolerance in an ordinary strain of rats that is not genetically susceptible to diabetes or obesity. Several aspects of our study design are unique and advantageous. First, our experimental period was longer than 10 months. The longer examination period enabled significant differences in body weights and glucose tolerance to develop between the two groups. Four previous studies had the same experimental period as the present study (Blakely *et al.* 1981; Tulp *et al.* 1991; Levi & Werman 1998; Chicco *et al.* 2003). However, only one study could demonstrate a significant weight gain in an ordinary strain of rats that was fed a sucrose-rich diet (Chicco *et al.* 2003). The second unique aspect of our study was the use of glucose tolerance tests.

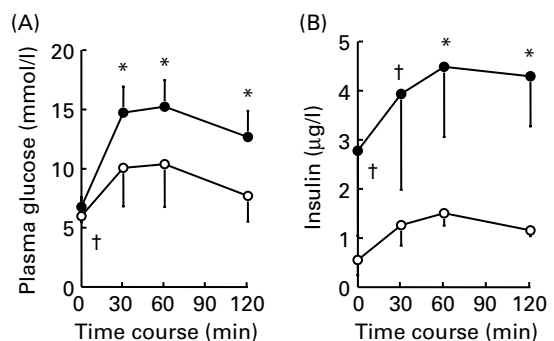


Fig. 5. Oral glucose tolerance test at 36 weeks of age. Glucose (1 g/kg) was loaded via oral catheter. (A), Plasma glucose concentrations in the sucrose group (●, *n* 16) were significantly higher than those in the control group (○, *n* 16) at fasting ($\dagger P < 0.05$), 30 min ($*P < 0.01$), 60 min ($*P < 0.01$) and 120 min ($*P < 0.01$) after loading. (B), Plasma insulin concentrations in the sucrose group (●, *n* 6) were significantly higher than those in the control group (○, *n* 6) at fasting ($\dagger P < 0.05$), 30 min ($\dagger P < 0.05$), 60 min ($*P < 0.01$) and 120 min ($*P < 0.01$) after loading. Values are means with their standard deviations depicted by vertical bars.

This allowed us to demonstrate that the mean 120 min post-load blood glucose concentration in the sucrose group was >11.1 mmol/l in 28-week-old rats and then reached approximately 12.5 mmol/l in 36-week-old rats. Oral glucose tolerance tests were performed in some previous studies (Blakely *et al.* 1981; Koyama *et al.* 1988; Stark *et al.* 2000). However, only one study could demonstrate the significantly higher mean 120 min post-load blood glucose concentration in fructose- or sucrose-loaded rats. Blakely *et al.* (1981) examined the oral glucose load (250 mg per 100 g body weight) at 3, 5, 7, 9 and 15 months in fructose-loaded rats. Although the serum glucose concentrations of the fructose-loaded rats were significantly higher than those of the control rats at some time-points, the mean 120 min post-load blood glucose concentrations in the fructose-loaded rats failed to reach 11.1 mmol/l. The third unique aspect of our study was that we used littermates so that the comparable groups could be considered genetically closer. This allowed easier detection of the influence of long-term sucrose-drinking, an environmental factor, on the incidence of glucose intolerance.

We should also discuss limitations of the present study. First, we should consider the circumstances that rats in the sucrose group had no choice but to drink 300 g/l sucrose water when we interpret the present results. This is not the same as the human situation. Moreover, 300 g/l sucrose water is three times more concentrated than the soft drinks we usually drink. Body fat gain, which is known to cause glucose intolerance in rats, might be unavoidable under these circumstances. Although we believe our results are meaningful, we should also note that the present study design does not separate the effects of sucrose-drinking from the effects of excess body fat. Hence, any source of excess energy, be it alcohol, milk, starch, protein or fat, might have the same effects. Second, IVGTTs were performed on rats at 8 and 16 weeks of age, although we were able to compare the two groups in the tests. It is well known that incretin responses in IVGTT are different from those in OGTT. We selected IVGTTs on the rats at these weeks of age in order to avoid the risk that the rat digestive organ might be injured by an oral catheter, as we planned long-term examination periods.

The results of most previous studies were consistent with our results regarding fasting plasma insulin in sucrose-drinking rats (i.e. increases in fasting plasma insulin concentrations). In the present study, fasting plasma insulin concentrations in the sucrose group increased to six times those of the control group at 12 weeks of age, although no difference in body weights was observed. Increases in fasting plasma insulin concentrations preceded greater weight gain in the sucrose group. Moreover, fasting plasma insulin concentrations continued to be elevated in the sucrose group after 12 weeks of age. We believe that the elevated insulin concentrations were caused by continuous sucrose-drinking, although we were unable to estimate the daily intake of sucrose water in each rat. When we examined plasma insulin concentrations from OGTTs, we found that these concentrations peaked in the sucrose group 30 min after loading at 28 weeks of age. At 36 weeks of age, the peak disappeared and hyperinsulinaemia was prolonged, accompanied with the progression of hyperglycaemia. These findings were very similar to those observed in patients with type 2 diabetes.

There are several possible explanations for the apparent hyperglycaemia observed in the sucrose group. First, weight gain plays an important role in the onset of diabetes, because obesity is a remarkably strong risk factor for diabetes in humans (Edelstein

et al. 1997). Second, as most previous studies have already demonstrated, sucrose-loading itself leads to glucose intolerance or hyperinsulinaemia in rats. Third, insulin secretion may be genetically weak in our rats. In the control group, the peak plasma insulin concentrations were detected at 60 min, not 30 min, after loading during the IVGTTs in 16-week-old rats and the OGTTs in 28- and 36-week-old rats. Fourth, sucrose-loading might accelerate the loss of islet β cells. Koyama *et al.* (1988) reported accelerated loss of islet β cells in sucrose-fed Goto-Kakizaki rats, a spontaneously diabetic model of non-insulin-dependent diabetes. Although we did not examine the volume density of islet β cells, we suspect that longer periods of sucrose-loading and progression of hyperglycaemia in the present study could accelerate the loss of islet β cells.

In human studies, a positive correlation between obesity and sugar intake was demonstrated in three previous studies (Ludwig *et al.* 2001; Raben *et al.* 2002; James *et al.* 2004). However, a positive relationship between sugar intake and the incidence of type 2 diabetes has not been demonstrated (Meyer *et al.* 2000; Janket *et al.* 2003).

The present results were quite different from the results of the human studies. There are several possible reasons for this discrepancy. First, dietary variation before the experimental period can be a confounding factor in human studies. Second, genetic homogeneity did not seem to be confirmed among the comparable groups in human studies. Although crossover study design could solve this problem, most of these studies had short experimental periods, ranging from 2 weeks to 3 months. Third, human epidemiologic studies are often characterized by poor dietary assessment methods (Nelson & Bringham, 1991). Fourth, accompanying decreases in fat intake might play an important role. Sugar intake and fat intake are generally reciprocally related in humans (Gibney, 1990). In the present study, the intake of the basal non-purified diet was significantly greater in the control group than in the sucrose group (data not shown). This means that fat intake was also significantly greater in the control group. Therefore, glucose intolerance in the sucrose group did not contribute to accompanying increases in fat intake. Fifth, extreme diets in which sucrose accounted for $>60\%$ of total calories (the highest contribution was 69%; Storlin *et al.* 1988) are often chosen in animal studies; whereas the highest contribution made by sucrose is approximately 33% (Reiser *et al.* 1981) in human studies. This difference between human and animal studies may make it difficult to compare results. The percentage of sucrose that was used in the present study is less than the highest percentage of sucrose in human studies.

In conclusion, we found that long-term sucrose-drinking causes increased body weight and glucose intolerance in normal male rats.

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