Tohoku J. Exp. Med., 1999, **189**, 59-70

Impairment of Glucose Tolerance in Normal Adults Following a Lowered Carbohydrate Intake

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WANG, P.-Y., KANEKO, T., WANG, Y., TAWATA, M. and SATO, A. *Impairment of Glucose Tolerance in Normal Adults Following a Lowered Carbohydrate Intake.* Tohoku J. Exp. Med., 1999, **189** (1), 59-70. —— Some normal people are falsely classified as having impaired glucose tolerance (IGT) if they are given an oral glucose tolerance test (OGTT) when their last meal contained very few carbohydrates. In this study, the duration of carbohydrate restriction was extended to one and three days and the relationship between the carbohydrate restriction and the glucose tolerance after an OGTT was examined. Two different groups of normal subjects were placed on high-carbohydrate (80% carbohydrates) and low-carbohydrate (10%) diets before an OGTT; one group for one day and the other for 3 days. None of the subjects showed impairment of glucose tolerance when placed on the high-carbohydrate regimens. In contrast, 3 of 12 subjects and 2 of 8 subjects placed on the low-carbohydrate diets for 1 and 3 days, respectively, were classified as having IGT. The impairment of glucose tolerance was invariably accompanied by an increase in the fasting plasma free fatty acid level. The longer the period of carbohydrate restriction, the severer was the glucose tolerance impairment. However, the number of subjects who were classified as having IGT did not depend on the duration of carbohydrate restriction. The impairment of glucose tolerance after carbohydrate restriction may be associated with the Randle effect, which is the activation of the glucose-free fatty acid cycle. ——— diabetes mellitus; glucose tolerance; oral glucose tolerance test; carbohydrate intake; free fatty acids

We evaluated the importance of carbohydrate intake in avoiding the misclassification of normal people by oral glucose tolerance tests (OGTT) as having diabetes or impaired glucose tolerance (IGT) (Kaneko et al. 1998). In this study, 12 young subjects ate breakfast and lunch, each of which had a normal carbohydrate content (60%). In the evening, half of the subjects ate a high-

Received June 7, 1999; revision accepted for publication September 14, 1999.

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carbohydrate meal containing 80% carbohydrate, and the other half ate a low-carbohydrate meal containing 10% carbohydrate. One week later, those who had eaten the high-carbohydrate evening meal were given the low-carbohydrate meal and vice versa. When given the low-carbohydrate meal, four subjects were classified as having IGT (120-minute postload glucose level >7.8 mM), while none of the subjects had IGT when given the high-carbohydrate meal. This study demonstrated that some normal people may be falsely classified as having IGT if they are given an OGTT when their last meal contained very few carbohydrates, even when the daily carbohydrate intake meets the WHO recommendation (>150 g/day) (WHO 1985).

In the present study, we extended the period of carbohydrate restriction to one and three days and examined the relationship between the restricted carbohydrate intake and the glucose tolerance after a 75-g glucose load.

Subjects and Methods

Subjects, dietary regimens and analyses

This study used two different experimental protocols: Experiment 1 involved a one-day test diet (three low-carbohydrate meals) and Experiment 2 a three-day test diet before an OGTT. The experiments were conducted with different subjects on different occasions. The materials and methods common to both experiments are described here. The subjects were undergraduate medical students at the Medical University of Yamanashi. A physical examination and routine laboratory tests indicated that they were all healthy. None had a family history of diabetes mellitus. The purpose and nature of the study were explained to all the subjects, and their written consent was obtained before participation.

Prior to each experiment, a dietary survey of all the subjects was conducted, using a 7-day weighted inventory questionnaire (Marr 1971; Fehily 1983), which was slightly modified in accordance with Japanese dietary habits, as described elsewhere (Nakajima et al. 1994). This survey confirmed that the nutritional intake of each subject was not greatly different from the typical Japanese intake in terms of carbohydrate, protein and fat intake (data not shown). Presently, the approximate intake of each macronutrient in Japan as a percentage of total calories is carbohydrate, 60%; protein, 15%; fat, 25% (Health Promotion and Nutrition Division 1994).

The test diets were high-carbohydrate (carbohydrate 80%; protein 15%; fat 5%) and low-carbohydrate (carbohydrate 10%; protein 25%; fat 65%) diets (Table 1). The diets were prepared and weighed in the kitchen of the University Hospital following a menu provided by a dietitian. The total daily caloric intake was fixed at 30 kcal $\cdot$ kg^{-1} $\cdot$ day^{-1} in each of the experiments. Meals, which were eaten at 08 : 00, 13 : 00 and 18 : 00, contained 20, 40 and 40% of the daily caloric intake, respectively. The subjects of each experiment were randomly divided into two groups and the subjects in each group consumed the assigned diets in a

TABLE 1. *An example of test diet menu for a subject whose body weight is 60 kg*

High-carbohydrate diet		Low-carbohydrate diet	
Food	Weight (g)	Food	Weight (g)
Breakfast		Breakfast	
Bread and bean jam	151	Salad	
Salad		Tomato	48
Cabbage	38	Celery	48
Carrot	8	Cucumber	48
Soy sauce	4	Mayonnaise	6
Orange juice	151	Boiled egg	60
		Cheese	24
Lunch		Lunch	
Cooked rice	265	Fried fish with white sauce	
Dashimaki (Egg crepe)		Flat fish	120
Egg	53	Starch	12
Sugar	8	Corn oil	12
Corn oil	2	White sauce	12
Grated *daikon* with soy sauce		Lettuce	36
Daikon (Radish)	23	Lightly fried tofu (bean curd)	
Soy sauce	2	Tofu	240
Nimono (Cooked vegetables)		Starch	12
Potato	76	Corn oil	24
Carrot	15	Lettuce	36
Onion	15	Radish sprout	6
Sugar	8	Soy sauce	6
Soy sauce	8	*O-hitashi* (Boiled greens with soy sauce)	
O-hitashi (Boiled greens with soy sauce)		Spinach	120
Spinach	45	Soy sauce	6
Soy sauce	4		
Lemon juice with honey	151		
Supper		Supper	
Cooked rice	265	Omelet	
Fish cooked with soy sauce and sugar		Egg	60
Flat fish	53	Minced beef	36
Soy sauce	8	Onion	24
Sugar	8	Corn oil	6
Nimono (Cooked vegetables)		Tomato ketchup	6
Daikon (Radish)	61	Sirloin steak	
Carrot	15	Butter	6
Soy sauce	8	Steak sauce	6
Sugar	8	Grapefruit	120
Grape juice	151		

cross-over design study with at least a one-week interval between tests. The investigators verified that the meals were consumed. The subjects were asked not to consume any other food or beverages except water and not to engage in strenuous physical activity during the test diet period. On the day of the OGTT, all the subjects reported that they had followed the instructions.

The fasting blood was collected between 09 : 00 and 09 : 30. The plasma was promptly separated and immediately submitted for free fatty acid (FFA) and triglyceride analyses. The remaining plasma was kept in ice-cold water. Plasma analyses for glucose (fasting plasma glucose, FPG), insulin (fasting plasma insulin, FPI), FFA, triglycerides and total cholesterol were performed using test kits purchased from Wako Pure Chemicals (Osaka: Glucose CII-Test, Glazyme Insulin-EIA Test, NEFA C-Test, Triglyceride E-Test and Cholesterol E-Test, respectively), with a spectrophotometer (Clinical Spectrophotometer 7010 with X-Y Autosampler, Hitachi, Tokyo). All of the analyses were performed in duplicate and completed within 2 hours of the last blood sampling.

For the OGTT, a lemon-flavored, carbonated solution (Toleran G75, Shimizu Pharmaceutical Co., Shizuoka) containing starch hydrolysates equivalent to 75 g glucose in 225 ml was taken orally between 09 : 40 and 10 : 20. Blood was sampled 30, 60 and 120 minutes after the glucose was ingested. The plasma glucose level of all three blood samples and the plasma insulin level of the first sample were measured using the same methods described above. An "insulinogenic index," defined as the ratio of the change in circulating insulin to the change in the corresponding glycemic stimulus, was calculated using the equation (30-minute plasma insulin-FPI)/(30-minute plasma glucose-FPG) (Seltzer et al. 1967). The results of the OGTT were classified into three categories (normal, impaired, or diabetic) using the 120-minute postload glucose value according to the WHO classification criteria (WHO 1985). The increment of plasma glucose following the glucose load was expressed in terms of the areas under the plasma glucose-time curve (AUC) for the time between when the fasting blood was drawn until the 120-minute postload blood sampling, using the trapezoidal rule.

The data were subjected to ANOVA using StatView 4.0 (Abacus Concepts, Berkeley, CA, USA). Student's paired and unpaired t-tests were used when there was a significant difference between the high- and low-carbohydrate diets and between the one- and three-day test dietary regimens, respectively. The 0.05 level of probability was used as the criterion of significance. No significant difference was detected between the male and female subjects in Experiment 2, so the pooled data for both sexes are given in the tables as mean $\pm$ S.D.

Experiment 1 (one-day study)

Twelve male volunteers (age, 21.9 ± 1.2 years; body weight, 64.4 ± 6.1 kg; height, 1.70 ± 0.03 m) participated in this experiment. They consumed high- and low-carbohydrate diets for one day in a cross-over design study with a one-week interval between tests. The carbohydrate intake for the high- and low-carbohydrate regimens was 6.00 and 0.75 g • kg^{-1} • day^{-1}, respectively.

Experiment 2 (three-day study)

Eight subjects consisting of 5 men (age, 21.6±1.5 years; body weight, 63.7±10.7 kg; height, 1.71±0.10 m) and 3 women (21.7±0.6 years; 50.8±2.3 kg; 1.61±0.02 m) volunteered for this experiment. They consumed high- and low-carbohydrate diets for 3 days in a cross-over design study with a two-week interval between tests. The carbohydrate intake for the high- and low-carbohydrate regimens was 6.00 and 0.75 g • kg^{-1} • day^{-1}, respectively.

Results

Experiment 1 (one-day study)

The FPG values, which were all within the normal range, were significantly higher ($p<0.05$) when the subjects were on the high-carbohydrate diets than when they were on the low-carbohydrate diets (Table 2). However, the postload glucose levels from 30 minutes through 120 minutes were always higher when the subjects were on the low-carbohydrate regimen than when they were on the high-carbohydrate regimen.

Three of the 12 subjects on the low-carbohydrate regimen were classified as

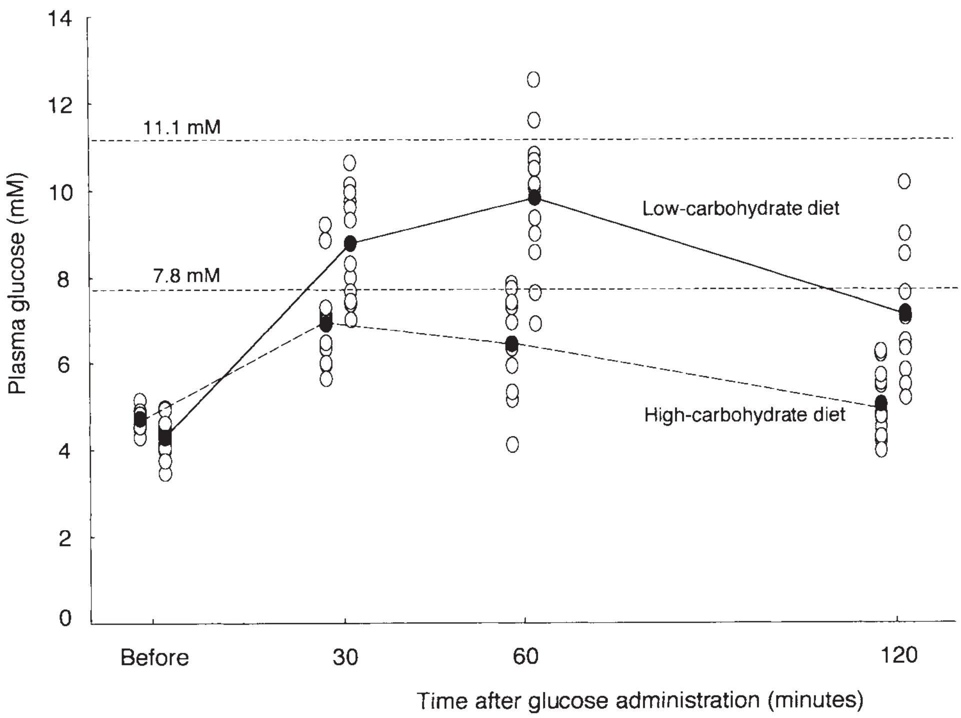

Fig. 1. Plasma glucose-time curves of individual participants during the OGTT after one day of test dietary regimen. Each participant is shown by the symbols connected with the same line. The open symbols are for plasma glucose levels after the low-carbohydrate intake and the closed symbols for those after high-carbohydrate intake.

TABLE 2. *Plasma glucose and insulin concentrations before and after a challenge with 75 g glucose*

Diets	Glucose (mM)				AUC (mM × min.)	Insulin (pM)		Insulinogenic index (pM/mM)
	Before	30 minutes	60 minutes	120 minutes		Before	30 minutes	
Experiment 1 (1-day test diet study)								
High	4.7 ±0.3	6.6 ±1.3	6.5 ±1.1	5.0 ±0.8	122 ±68	61 ±16	332 ±80	142 ±68
Low	4.3 ±0.4[a]	8.8 ±1.3[a]	9.8 ±1.6[a]	7.1 ±1.5[a]	400 ±112[a]	64 ±11	350 ±82	70 ±33[a]
Experiment 2 (3-day test diet study)								
High	4.3 ±0.5	6.9 ±1.6	6.6 ±0.9	5.0 ±1.1	162 ±64	63 ±9	303 ±118	136 ±125
Low	3.5 ±0.4[a,b]	8.0 ±1.8[a]	9.1 ±1.4[a]	7.1 ±2.9[a]	434 ±129[a]	73 ±21	298 ±161	48 ±42[a]

Values are the mean ± S.D. for 12 (Experiment 1) and 8 (Experiment 2) subjects. High, high-carbohydrate diet; Low, low-carbohydrate diet. [a]Significantly different from High in the same experiment ($p < 0.05$). [b]Significantly different from Low in Experiment 1 ($p < 0.05$).

having IGT (120-minute postload glucose levels: 10.2, 9.0, and 8.5 mM) (Fig. 1). In contrast, all of the subjects, when they were placed on the high-carbohydrate regimen, had normal glucose tolerance. The postload glucose levels of the subjects who were classified as having IGT were 6.2, 5.5, and 4.2 mM, respectively, when they were on the high-carbohydrate regimen.

No significant differences were observed in either the FPI or the 30-minute postload insulin level between the high- and low-carbohydrate diets (Table 2). However, the insulinogenic index after the low-carbohydrate diet was almost one-half that after the high-carbohydrate diet.

The most significant change in the fasting plasma lipids was found in the FFA level (Table 3). The level following the low-carbohydrate diet was almost twice as high as that after the high-carbohydrate diet.

Experiment 2 (three-day study)

The FPG values were significantly higher ($p < 0.05$) when the subjects were kept on the high-carbohydrate diets than when they were kept on the low-carbohydrate diets, although all of the values remained within the normal range (Table 2). The average FPG value after three days of carbohydrate restriction was significantly lower ($p < 0.05$) than that after one day of carbohydrate restriction.

The postload plasma glucose values (30 minutes through 120 minutes) were significantly higher after the low-carbohydrate diet than those after the high-carbohydrate diet (Table 2).

TABLE 3. *FFA, triglycerides and total cholesterol in the fasting plasma*

Diets	FFA (mM)	Triglycerides (mM)	Total cholesterol (mM)
Experiment 1 (1-day test diet study)			
High	0.40±0.15	0.90±0.23	4.20±0.40
Low	0.78±0.33[a]	0.71±0.30[a]	4.32±0.39
Experiment 2 (3-day test diet study)			
High	0.48±0.09	1.01±0.36	4.55±0.39
Low	1.51±0.47[a,b]	0.71±0.19[a]	5.43±0.83[a,b]

Values are the mean±S.D. for 12 (Experiment 1) and 8 (Experiment 2) subjects. High, high-carbohydrate diet; Low, low-carbohydrate diet. [a]Significantly different from High in the same experiment ($p<0.05$). [b]Significantly different from Low in Experiment 1 ($p<0.05$).

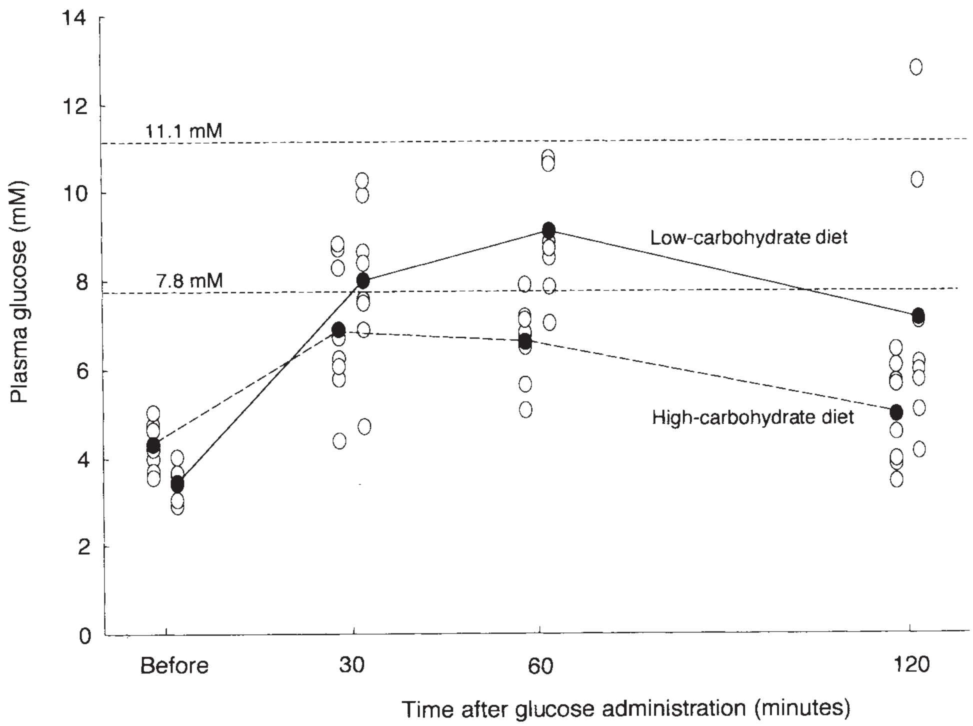

Fig. 2. Plasma glucose-time curves of individual participants during the OGTT after three days of test dietary regimen. Each participant is shown by the symbols connected with the same line. The open symbols are for plasma glucose levels after low-carbohydrate intake and the closed symbols for those after high-carbohydrate intake.

Of the 8 subjects (5 men and 3 women) placed on the low-carbohydrate diet, one woman was classified as diabetic (120-minute postload glucose level, 12.8 mM) and one man as having IGT (10.2 mM). None of the subjects showed the impairment of glucose tolerance when they were placed on the high-carbohydrate diet (Fig. 2). The two subjects who were classified as diabetic and as having IGT after the carbohydrate-restricted diet had normal glucose tolerance (the postload glucose levels; 6.4 and 6.1 mM, respectively) when they were placed on the high-carbohydrate diet. The highest 120-minute postload glucose level after the three-day carbohydrate restriction (12.8 mM; diabetic) was much higher than the highest level after the one-day carbohydrate restriction (10.2 mM; IGT).

No significant difference was observed in either the FPI or the 30-minute postload insulin level between the two carbohydrate regimens (Table 2). However, the insulinogenic index was significantly lower after the low-carbohydrate diet than after the high-carbohydrate diet. The average value of the index was lower after the three-day carbohydrate restriction (48 pM/mM) than after the one-day carbohydrate restriction (70 pM/mM), although the difference was not statistically significant ($p > 0.2$).

The most striking change in the fasting plasma lipids caused by the three-day intake of low-carbohydrate diet was the elevation of FFA level (Table 3), which was almost three-times higher than it was after the high-carbohydrate diet. The average FFA value after three days of carbohydrate restriction (1.51 mM) was about twice as high as that after one day of carbohydrate restriction (0.78 mM).

Discussion

For over 60 years, it has been known that reduced carbohydrate intake before an OGTT adversely affects glucose tolerance of normal people. Himsworth (1935) studied the effect of 7-day diets containing various amounts of carbohydrate and fat on the glucose tolerance of healthy men, using a 50-g OGTT. He found that glucose tolerance improved with a change from a low-carbohydrate/high-fat diet to a high-carbohydrate/low-fat diet. This improvement was affected neither by changes in the total calories, by the carbohydrate/fat ratio in the diet, nor by the fat or protein content of the diet, but solely by the amount of carbohydrate in the diet. In Himsworth's study, a marked decrease in glucose tolerance was observed when the daily diet contained 50 g of carbohydrate.

Conn (1940) stressed the necessity of dietary preparation before an OGTT. Nine subjects were placed on a daily diet of 3000 kcal containing 300 g of carbohydrate for three days, and an OGTT (glucose load, 1.75 g/kg) was performed. All of the glucose tolerance curves were normal. Next, all of the subjects were placed on a daily diet of 1600 kcal containing 20 g of carbohydrate for five days and were given an OGTT. They all showed a marked decrease in glucose tolerance and three subjects were falsely diagnosed as diabetic. However, the glucose tolerance returned to normal after the subjects returned to a high-carbohydrate diet. Based on these results, Conn (1940) recommended that everyone should have a preparatory diet of 3000 kcal/day, containing 300 g of carbohydrate, for at least three days before an OGTT.

To confirm the experimental results obtained by Himsworth (1935) and by Conn (1940), Wilkerson et al. (1960) evaluated the glucose tolerance of 42 men and

15 women placed on a daily diet containing 50 g of carbohydrate for three (men) or seven (women) days, with a 100-g OGTT. From their results, Wilkerson and the colleagues concluded that antecedent preparation of diets with as little as 50 g of carbohydrate had a negligible effect on the diagnostic results of an OGTT. However, this conclusion conflicts with their observation of a high frequency of glycosuria after the low-carbohydrate diet. One hour after the glucose challenge, glycosuria (Clinitest) was found in 16.3% of the men placed on the 50-g carbohydrate diet for three days, although the average glucose level was 116 ± 30.3 mg/100 ml (6.4 ± 1.7 mM) and no subject reached a level of 170 mg/100 ml (9.4 mM). The glycosuria observed one hour after the glucose load was evidently caused by the elevated postload glucose concentration in the blood, because none of the subjects had glycosuria in their fasting urine.

However, the Committee on Statistics of the American Diabetes Association (1969) attached great importance to the report of Wilkerson et al. (1960). They stated that: "Severe carbohydrate restriction has long been known to produce decreased carbohydrate tolerance in normal individuals, and it has been proposed that dietary preparation consist of ingestion of a food intake containing 300 g of carbohydrate for each of three days prior to the test. Wilkerson et al. (1960) showed, however, that daily carbohydrate intakes of as low as 50 g caused only slightly decreased glucose tolerance. For this reason, and because 300 g of carbohydrate may be unpalatable to some persons, it is recommended that there be a minimum carbohydrate intake of 150 g for each of the three days preceding the test provided that the subject was consuming a normal diet prior to this period." The National Diabetes Data Group of the National Institute of Health (1979) and WHO (1985) have since adopted this recommendation of the American Diabetes Association. The provisional report of a WHO consultation has followed the same recommendation (Alberti and Zimmet 1998).

We have demonstrated that some normal people may be misclassified as having IGT if they are given an OGTT when their last meal contained very few carbohydrates (Kaneko et al. 1998), even when the daily carbohydrate intake meets the WHO recommendation (>150 g/day) (WHO 1985). Before OGTT, patients are usually instructed to "fast" from 21 : 00 the previous day. Hence, some may refrain from consuming carbohydrates at the last meal in anticipation of an "accurate diagnosis." We should know that unrestricted carbohydrate intake before the test is important in avoiding an error in diabetes diagnosis.

In our present study, where the duration of carbohydrate restriction was extended to one or three days, the impairment of glucose tolerance after a 75-g glucose load was much severer after three days of carbohydrate restriction. The highest 120-minute postload glucose levels attained in the three-day and one-day studies were 12.8 mM and 10.2 mM, respectively. In our previous study (Kaneko et al. 1998), where 12 subjects only consumed a low-carbohydrate evening meal, the highest 120-minute postload glucose level was 8.7 mM. Together, these

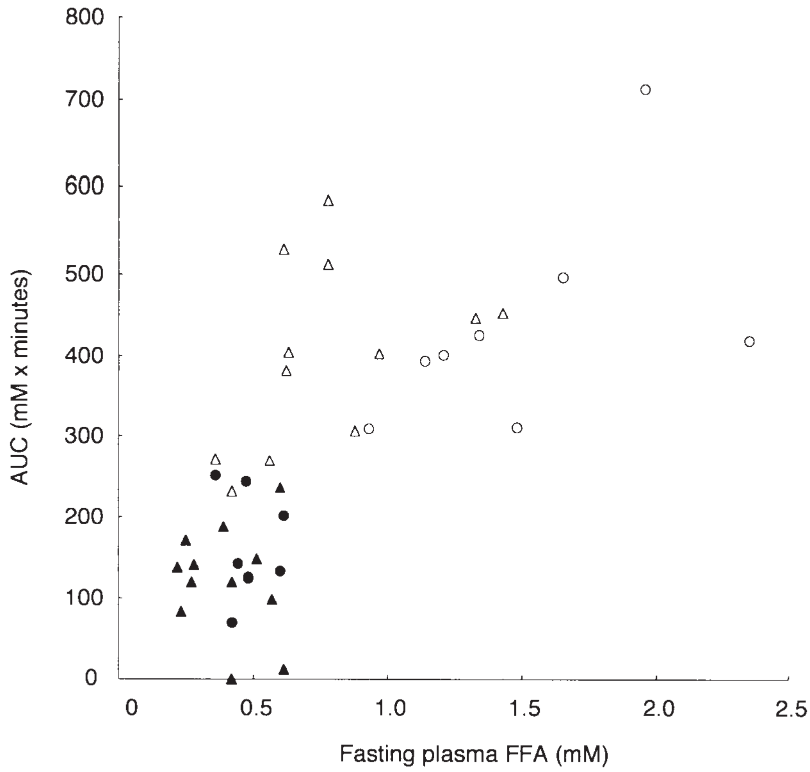

Fig. 3. Relationship between the FFA level in the fasting plasma and the AUC of plasma glucose ($r=0.697$, $n=40$).
▲, High-carbohydrate (one day); △, Low-carbohydrate (one day); ●, High-carbohydrate (three days); ○, Low-carbohydrate (three days).

findings suggest that the longer the period of carbohydrate restriction, the severer the impairment of glucose tolerance.

On the other hand, the number of subjects who were classified as having IGT seemed not to depend on the duration of carbohydrate restriction. Four (33.3%) of the 12 subjects placed on the evening meal containing low carbohydrates (Kaneko et al. 1998), three (25.0%) of the 12 subjects on the low-carbohydrate regimen for one day (Fig. 1), and two (25.0%) of the 8 subjects on the low-carbohydrate regimen for three days (Fig. 2) were classified as IGT or diabetic. These findings suggest that about 30% of normal, young Japanese may be specifically sensitive to carbohydrate restriction in terms of glucose tolerance and that a one-meal test, i.e., a low-carbohydrate in the last meal before an OGTT, effectively screens the people sensitive to carbohydrate restriction. It should be determined whether the decreased glucose tolerance after carbohydrate restriction is associated with a genetic predisposition to diabetes mellitus.

The impairment of glucose tolerance after restricted carbohydrate intake was invariably accompanied by an increase in the fasting plasma FFA level (Table 3). The increase in the FFA level was also observed after a one-meal carbohydrate restriction (Kaneko et al. 1998). This suggests that the impairment is associated with the Randle effect (Randle et al. 1963), which is the activation of the glucose-free fatty acid cycle.

According to Randle (1993, 1995) and Randle et al. (1994), FFA adversely affects glucose homeostasis by inhibiting glucose oxidation, by stimulating hepatic glucose production, and by inhibiting the secretion of insulin by β-cells in the pancreatic islets of Langerhans in response to glucose. All of these effects of FFA on glucose metabolism may be associated with the decreased glucose tolerance after carbohydrate restriction. The FFA level in the fasting plasma was positively correlated with the AUC of plasma glucose (Fig. 3; $r=0.70$, $n=40$, $p<0.05$).

The insulinogenic index decreased after a restricted carbohydrate intake (Table 2) and the index was negatively correlated with the FFA level in the fasting plasma ($r=-0.45$, $n=40$, $p<0.05$). This suggests that proportionally less insulin is released in response to the glycemic stimulus after carbohydrate restriction and that the decreased insulin secretion is associated with the increased FFA level. However, further studies are needed to clarify the mechanism underlying the impairment of glucose tolerance induced by restricted carbohydrate intake.

Acknowledgments

We thank all the subjects who volunteered for this study. We also thank Mr. Iwao Koyama and Mr. Kaoru Asami for providing us with the test diets. This work was supported by a Grant-in-Aid (09470111) from the Ministry of Education, Science, Sports and Culture of Japan.

References

1) Alberti, K.G.M.M. & Zimmet, P.Z. (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet. Med.*, **15**, 539-553.

2) Committee on Statistics of the American Diabetes Association (1969) Standardization of the oral glucose tolerance test. Report of the Committee on Statistics of the American Diabetes Association. *Diabetes*, **18**, 299-307.

3) Conn, J.W. (1940) Interpretation of the glucose tolerance test. The necessity of a standard preparatory diet. *Am. J. Med. Sci.*, **199**, 555-564.

4) Fehily, A.M. (1983) Epidemiology for nutritionists: 4. Survey methods. *Human Nutrition Applied Nutrition*, **37A**, 419-425.

5) Health Promotion and Nutrition Division, Health Service Bureau, Ministry of Health and Welfare of Japan (1994) *The 1994 National Nutrition Survey in Japan*. Dai-ichi Shuppan, Tokyo. (in Japanese)

6) Himsworth, H.P. (1935) Dietetic factor determining glucose tolerance and sensitivity to insulin of healthy men. *Clin. Sci.*, **2**, 67-94.

7) Kaneko, T., Wang, P.-Y., Tawata, M. & Sato, A. (1998) Low carbohydrate intake before oral glucose-tolerance tests. *Lancet*, **352**, 289.

8) Marr, J.W. (1971) Individual dietary surveys: Purposes and methods. *World Rev. Nutr. Diet*, **13**, 105-164.

9) Nakajima, T., Ohta, S., Fujita, H., Murayama, N. & Sato, A. (1994) Carbohydrate-related regulation of the ethanol-induced increase in serum g-glutamyl transpeptidase activity in adult men. *Am. J. Clin. Nutr.*, **60**, 87-92.

10) National Diabetes Data Group of National Institute of Health (1979) Classification

and diagnosis of diabetes mellitus and other categories of glucose intolerance. *Diabetes*, **28**, 1039–1057.

11) Randle, P.J., Garland, P.B., Hales, C.N. & Newsholme, E.A. (1963) The glucose fatty acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetic mellitus. *Lancet*, I, 785–789.
12) Randle, P.J. (1993) Glucokinase and candidate genes for type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*, **36**, 269–275.
13) Randle, P.J., Priestman, D.A., Mistry, S.C. & Halsall, A. (1994) Glucose fatty acid interactions and the regulation of glucose disposal. *J. Cell. Biochem.*, **55**, S1–S11.
14) Randle, P.J. (1995) Metabolic fuel selection: General integration at the whole-body level. *Proc. Nutr. Soc.*, **54**, 317–327.
15) Seltzer, H.S., Allen, E.W., Herron, A.L., Jr. & Brennan, M.T. (1967) Insulin secretion in response to glycemic stimulus: Relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J. Clin. Invest.*, **46**, 323–335.
16) WHO (1985) *Diabetes Mellitus: Report of a WHO study group (Technical report series, No. 727)*, WHO, Geneva.
17) Wilkerson, H.L.C., Hyman, C., Kaufman, M., McCuistion, A.C. & Francis, J.O. (1960) Diagnostic evaluation of oral glucose tolerance tests in nondiabetic subjects after various levels of carbohydrate intake. *N. Engl. J. Med.*, **262**, 1047–1053.