givalis (IgG titer > 310), and that the degree of carotid stenosis, but not IMT in plaque-free segments, characterized the patients with a high IgG titer against porphyromonas gingivalis as compared with those with normal a IgG titer. The patients studied were unique in that they were non-obese and were well controlled in terms of HbA_{1c} (mean HbA_{1c} 7.1%) and BP (mean BP, 130/76 mm Hg). Moreover, they had no evidence of CHD, ischemic stroke, or chronic renal failure. Of particular interest is that the degree of carotid stenosis was 8.9% ±1.4% and that the comparison was made between the groups with 12.0% and 5.5% (Table 1). To the best of our knowledge, however, carotid ultrasonography was not reported in type 2 diabetic patients with very early stages of carotid atherosclerosis, such as in our present study. Thus, the mechanism by which the very early stages of carotid atherosclerosis occurs in type 2 diabetic patients is not known, but it may be hypothesized that porphyromonas gingivalils infection is one of the factors responsible for the early onset of carotid atherosclerosis in non-obese Japanese type 2 diabetic patients. It is well known that carotid atherosclerosis is important in view of its relationship to cerebrovascular ischemic diseases and coronary atherosclerosis.[2]

Whereas porphyromonas gingivalis infection is a subclinical inflammation, there are some findings suggesting that subclinical infection is associated with atherosclerosis in man. Elevated levels of CRP, although still for the most part in the healthy reference range, have been shown to be associated with an increased risk of future CHD events.[4-6] Some cross-sectional and case-control studies have reported elevated antibody titers directed against *Chlamydia pneumoniae*, *Helicobacter pyroli*, and cytomegalovirus among those with prevalent heart disease.[7]

Thus, porphyromonas gingivalis infection seems to be associated with the early stage of atherosclerosis in non-obese Japanese type 2 diabetic patients.

Finally, carotid atherosclerosis was not significantly associated with an abnormal lipid profile, such as high concentrations of LDL cholesterol in the present study. The reason is unclear, but it may be due to the clinical characteristics studied. Our patients were all non-obese Japanese type 2 diabetic patients. Indistinct from white populations, non-obese Japanese type 2 diabetic patients are unique in that they are divided into 2 variants: one with normal insulin sensitivity and the other with insulin resistance.[15-19] Non-obese Japanese type 2 diabetic patients with insulin resistance are characterized by higher BMI, higher triglycerides, higher remnant-like particle cholesterol, and lower HDL cholesterol levels as compared with those with normal insulin sensitivity. Our patients studied were unique in that they were well controlled in terms of BMI, HbA_{1c}, BP, LDL cholesterol, triglycerides, total cholesterol, and HDL cholesterol. Furthermore, our patients had no evidence of cardiovascular disease, ischemic stroke, or chronic renal failure.[20] Therefore, an association between carotid atherosclerosis and conventional risk factors including LDL cholesterol would be more significant among obese type 2 diabetic patients who has abnormal lipid profile and/or an evidence of cardiovascular disease, ischemic stroke, or chronic renal failure. Alternatively, the diabetic state per se is such a powerful factor on carotid atherosclerosis that the effect of other risk factors is masked. Mohan et al[21] recently demonstrated that diabetes and age, but not conventional risk factors, are the most important risk factors associated with increased IMT in South Indian diabetic patients with a BMI of 24.5 kg/m^2.

In summary, although our present study was performed among the limited patients who were well controlled in terms of BMI, HbA_{1c}, BP, LDL cholesterol, triglycerides, total cholesterol, and HDL cholesterol, porphyromonas gingivalis infection seems to be associated with an early stage of atherosclerosis in non-obese Japanese type 2 diabetic patients.

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Effects of an aldose reductase inhibitor on gastroenteropathy in streptozotocin-diabetic rats

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Abstract

We investigated the effects of epalrestat, an aldose reductase inhibitor (ARI), on gastric emptying, fecal water content, and electrolyte transport in distal colon in streptozotocin (STZ)-induced diabetic rats. We measured gastric emptying time by acetaminophen method and short-circuit-current (Isc) in colonic mucosa using an Ussing chamber. The Isc in response to electric-field-stimulation (EFS) was decreased in untreated rats due to suppression by Cl^- secretion. ARI treatment alleviated this suppression (2.7 ± 0.6 vs. 7.4 ± 1.1 μA/0.38 cm^2 at 8 weeks after treatment, 1.1 ± 0.2 vs. 7.0 ± 1.0 at 12 weeks after treatment, $P < 0.05$). In addition, the percentage of fecal water content in untreated rats was significantly lower than in ARI-treated rats (58.0 ± 2.0 vs. 67.6 ± 0.8% at 8 weeks, 56.9 ± 2.1 vs. 63.4 ± 1.4 at 12 weeks, $P < 0.05$). From STZ injection to 8 weeks, the serum levels of acetaminophen in the diabetic rats were significantly lower than in controls, indicating delayed gastric emptying. At 12 weeks in the diabetic rats treated with ARI, the serum levels of acetaminophen were significantly higher than in the untreated diabetic rats (6.6 ± 0.4 vs. 3.5 ± 0.5 μg/ml, $P < 0.05$). ARI-treatment ameliorated delayed gastric emptying without improving glycemic control. These findings show that ARI partially prevented progression of impaired gastric emptying, ion transport, and water transport, and suggest that epalrestat might be useful in the treatment of diabetic gastroenteropathy.

Keywords: Diabetes mellitus; Aldose reductase inhibitor; Gastroenteropathy

1. Introduction

Diabetes mellitus is associated with gastrointestinal disturbances such as nausea, constipation and diarrhea [1–3]. Previous studies have indicated that these disturbances are due to dysfunction of the autonomic nervous system [4,5]. Although the cause of diabetic autonomic neuropathy remains unknown, evidence suggests the involvement of the polyol pathway, in which glucose is metabolized to sorbitol and fructose by aldose reductase and sorbitol dehydrogenase, respectively, [6,7]. Epalrestat, an aldose reductase inhibitor (ARI),

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is widely used in Japan to prevent the progression of diabetic neuropathy [8]. Nakamura et al. [9] reported that ARI treatment improves bowel motility in patients with type 2 diabetes mellitus, but the mechanism is not understood. As the association of diabetes mellitus with reduced in gastrointestinal motility and ion transport has been reported [10–12], it is important to determine the effect of ARI on gastric emptying and colonic ion transport. We have examined the effects of epalrestat on gastric emptying time and ion transport in the distal colon in streptozotocin (STZ)-induced diabetic rats. Gastric emptying time was evaluated using the acetaminophen method [13], and mucosal ion transport by measurement of short-circuit-current (Isc) in an Ussing chamber.

2. Materials and methods

2.1. Animals

In all experiments, young adult male Wistar rats weighing 250±3.5 g were used. The rats were housed in an air-controlled (temperature 25±2 °C and 50% humidity) room with 12-h light:12-h dark cycle. Diabetes was induced by the administration of a single intraperitoneal injection of STZ (60 mg/kg body weight) and the control rats were injected with physiologic saline. Three days after STZ injection, the blood glucose levels were determined, and animals with blood glucose levels over 16.7 mmol/l were considered to have diabetes mellitus. The diabetic rats were divided into two groups. Untreated rats (referred as to DM rat, n = 56; 32 rats for ion transport study, 24 rats for gastric emptying) were given standard rat chow (Oriental Yeast, Osaka, Japan), and ARI-treated rats (referred as to DM+ARI rat, $n = 56$ divided as above) were given standard rat chow containing 0.04% epalrestat (Oriental Yeast and Ono Pharmaceuticals Co., Osaka, Japan). Insulin-treated rats (referred as to DM+INS rat, $n = 16$) were started on daily doses of ultralente insulin (6–26 U/day: Shimizu Pharmaceutical, Shizuoka, Japan) designed to maintain blood glucose levels between 5.6 and 11.1 mmol/l. Insulin therapy was started 3 days after STZ injection and was maintained during the experiment. Hotta et al. has reported that standard rat chow containing 0.04% epalrestat corresponds to oral administration of epalrestat at the dose of 50 mg/kg body weight in STZ-diabetic rats [14]. Sasaki et al. has reported that oral administration of epalrestat at the dose of 50 mg/kg body weight ameliorated myelinated fiber atrophy in STZ-diabetic rats [15]. Age and weight-matched rats were used as controls (referred as to Control rats, $n = 56$). ARI treatment was begun on the day of STZ injection and continued for 12 weeks.

2.2. Short-circuit current (Isc) measurement

The stripped colonic mucosa(submucosal plexus intact) was mounted vertically between temperature (37 °C)-controlled Ussing-type chambers (10 ml each) with an exposed area of 0.38 cm^2 [16,17]. Ringer's solution, which contained (mM), NaCl 115, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25, K_2HPO_4 2.4, KH_2PO_4 0.4 and glucose 10, was used as the standard bathing solution. All bathing solutions were gassed with a mixture of 95% O_2/5%CO_2, resulting in a pH of 7.4. All drugs were applied to the serosal bathing solution from the stock solution.

Isc was measured using an automatic voltage clamping device (Nihon Kohden CEZ-9100, Tokyo, Japan) that compensates for resistance of the solution between the potential measuring electrodes. The transepithelial potential was recorded through 3 M KCl–agar bridges connected to a pair of calomel half cells. The transepithelial current was applied across the tissue via a pair of Ag/AgCl electrodes that was kept in contact with the mucosal and serosal bathing solution using a pair of 3 M KCl–agar bridges. The Isc is referred to as negative when current flows from mucosa to serosa. Electrical properties of the tissues attained stable value within 30 min after start of preincubation. In Cl^- free solution, gluconate was substituted for Cl^-.

Parameters for the stimulus were as follows for trains of pulses: pulse duration, 0.1 ms; train duration, 5 s; frequency, 10 Hz; voltage, 50 V. After the initial Isc in response to electric-field-stimulation (EFS) was determined, the neurotoxin

tetrodotoxin (TTX, 5 μM) and the muscarinic cholinergic agonist carbachol (CCH, 100 μM) were added to the serosal reservoir to determine whether the disordered ion transport was derived from nervous dysfunction in the submucosal plexus or from epithelial dysfunction.

2.3. Estimation of blood glucose levels and fecal water content

Blood glucose levels were estimated by sampling from the tail vein of the rats with Novo-assist (LIFE SCAN Inc, Milpitas, CA, USA). The final estimate of blood glucose was carried out after the animals were sacrificed. The fecal water content was calculated as follows: (fecal net weight-fecal dry weight after freeze-dried)/fecal net weight (%). Body weights and blood glucose levels were measured at 8 and 12 weeks after STZ injection. The percentage of fecal water content was measured at 8 and 12 weeks.

2.4. Measurement of gastric emptying

The acetaminophen method [13] was used. Before the experiments, rats were fasted for 18 h with free access to water. Acetaminophen is very poorly absorbed from the stomach, but almost completely and rapidly absorbed from the small intestine [18]. Following oral administration, serum acetaminophen levels are closely correlated with gastric emptying [13]. Since serum acetaminophen levels at 30 min have been reported to represent overall gastric emptying time [13], the rats were killed under ether anesthesia, and carotid blood was collected 30 min after the administration of acetaminophen (20 mg) in 2 ml of meal (prepared by suspending food—ground to powder—in distilled water at a 50% concentration). The serum acetaminophen levels were measured in duplicate by fluorescence polarization immunoassay using a TDx analyzer (Abbott Laboratories, Chicago, IL, USA).

2.5. Drugs and materials

STZ, carbachol, atropine, and guanethidine were purchased from Wako Chemicals (Osaka, Japan). TTX was from Sankyo Pharmaceuticals (Tokyo, Japan). Standard rat chow was from Oriental Yeast and rat chow containing 0.04% epalrestat was from Oriental Yeast and Ono Pharmaceuticals. All other chemicals were of reagent grade.

2.6. Statistical analyses

Differences between Control rats and DM rats were analyzed by Student's t-tests. Differences between DM rats and DM+ARI rats were analyzed by Student's t-tests. P values of <0.05 were considered statistically significant. All values are expressed as mean±standard error of the mean (S.E.M., n = number).

2.7. Ethical considerations

All studies were performed in the laboratories of the Department of Diabetes and Clinical Nutrition, Kyoto University, in accordance with the Declaration of Helsinki.

3. Results

3.1. Body-weight, blood glucose level and fecal water content

Control rats gained significant weight during the experiment (437.5±5.3 g at 8 weeks, 460.0±6.0 g at 12 weeks, $P<0.01$). In contrast, the diabetic animals with or without epalrestat treatment gained only a little weight (DM+ARI rats: 287.5±2.5 g at 8 weeks, 291.3±1.3 g at 12 weeks) (DM rats: 292.5±2.5 g at 8 weeks, 290.0±2.7 g at 12 weeks). Blood glucose levels were significantly higher in both diabetic groups compared with control rats. There was no differences in body weight or blood glucose level between the two diabetic groups. The ratio of fecal water to fecal solids in the DM rats was significantly lower than in the Control rats at 8 and 12 weeks after STZ treatment. There was no significant difference in fecal water to fecal solids ratio between the DM+ARI and Control rats (summarized in Table 1).

Table 1
Effect of ARI treatment on body weight, blood glucose and fecal water content in control and STZ-induced diabetic rats at 8 and 12 weeks

Group	Body weight (g)		Blood glucose (mM)		Fecal water content (%)	
	8 Weeks	12 Weeks	8 Weeks	12 Weeks	8 Weeks	12 Weeks
Control	437.5±5.3	460.0±6.0	4.4±0.2	4.5±0.2	68.2±1.0	67.9±0.9
DM+ARI	287.5±2.5*	291.3±1.3*	24.3±0.5*	25.1±0.6*	67.6±0.8	63.4±1.4
DM	292.5±2.5*	290.0±2.7*	24.6±0.5*	25.1±0.7*	58.0±2.0*#	56.9±2.1*#

Control rats gained significant during the experiment. The diabetic animals with or without epalrestat treatment gained only a little weight. Blood glucose levels were significantly higher in both diabetic groups compared with the control rats. There were no differences in body weight or blood glucose levels between the two diabetic groups. The ratio of fecal water to fecal solids in the DM rats significantly decreased compared with the control rats at 8 and 12 weeks after STZ treatment, but did not significantly decrease in the DM+ARI rats. All values are expressed as means±S.E.M. ($n=8$ for each group). DM+ARI and DM represent ARI-treated and untreated diabetic rats, respectively. Control represent ARI-untreated non-diabetic rats, respectively. *, $P<0.05$ vs. control rats; #, $P<0.05$ vs. DM+ARI rats.

3.2. Isc measurement

3.2.1. Control rats

Fig. 1 shows the Isc in response to EFS in control tissue at 8 weeks. The basal Isc in control rats was identical at 8 and 12 weeks (8 weeks; 7.9± 1.1 μA/0.38 cm^2, 12 weeks; 7.8±1.2 μA/0.38 cm^2). In the absence of TTX after 5 s with electric stimulation, the Isc increased rapidly to a peak in 30 s and then returned to base line within 2–3 min. In the Cl^--free solution, EFS did not evoke an increase in Isc, indicating that Isc in response to EFS was due to an increase of electrogenic Cl^- secretion (data not shown). In the presence of TTX, after 5 s of stimulation, the Isc in response to EFS did not increase, but the addition of CCH then induced the similar increase in Isc (8 weeks; 12.0±1.6 μA/0.38 cm^2, 12 weeks; 12.1±1.7 μA/ 0.38 cm^2). In the absence of TTX, CCH alone induced an increase in Isc similar to that induced by EFS (8 weeks; 12.5±1.8 μA/0.38 cm^2, 12 weeks; 12.0±1.4 μA/0.38 cm^2).

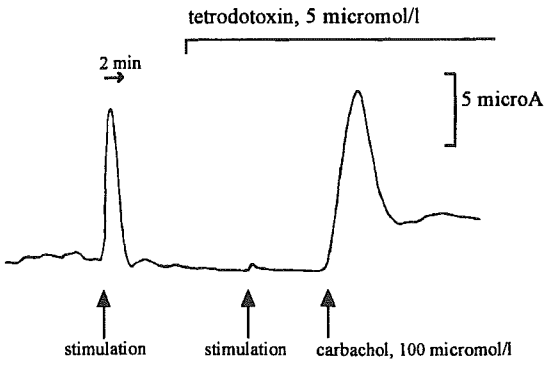

Fig. 1. Representative trace showing Isc in response to EFS in colonic mucosa of control rat. In the absence of TTX after 5 s with electric stimulation, Isc increased rapidly to a peak in 30 s and then returned to base line within 2–3 min. In the presence of TTX after 5 s stimulation, Isc in response to EFS did not increase, but addition of CCH then induced a similar increase in Isc.

CCH acting through muscarinic receptors on epithelial cells caused an increase in Isc which was unaffected by pretreatment with TTX, a neuronal conduction-blocker. Guanethidine(5 μM), an adrenergic blocker, did not affect Isc in response to EFS (data not shown). Atropine (1 μM) decreased the Isc response to EFS by 40%. This indicates that the Isc response to EFS was derived partly from cholinergic nerve function and partly from peptidergic nerve function.

3.3. Diabetic rats

In the diabetic groups, basal Isc was identical to that in the control rats (8 weeks; 7.5±1.3 μA/0.38 cm^2, 12 weeks; 7.8±1.2 μA/0.38 cm^2), and the elicited Isc in response to EFS was decreased compared with the control rats at 8 and 12 weeks (Fig. 2). Atropine (1 μM) decreased the Isc response to EFS by 40%. However, the Isc elicited by EFS in DM+ARI rats was higher than in DM

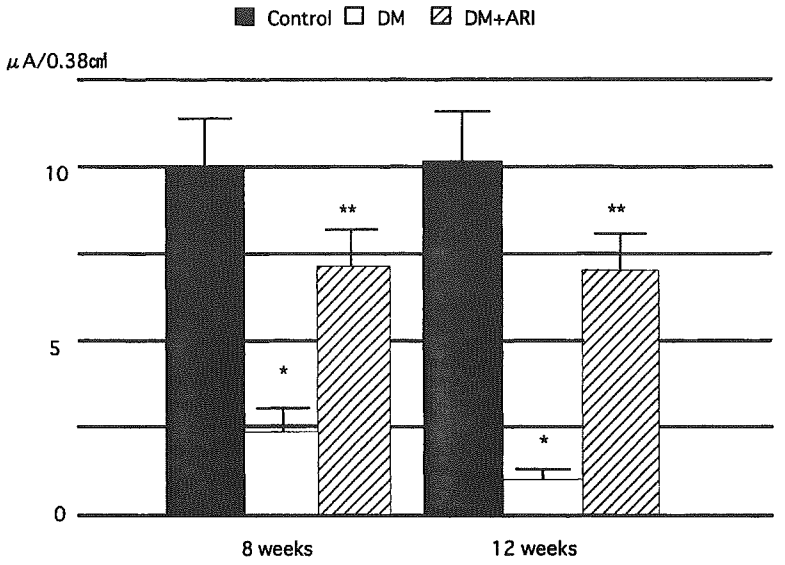

Fig. 2. Comparison of increase in Isc in response to EFS in rat colonic mucosa between groups at 8 and 12 weeks after STZ-injection. In DM rats, elicited Isc in response to EFS decreased when compared with control rats at 8 and 12 weeks. However, Isc elicited by EFS in DM+ARI rats was higher than in DM rats. Data are shown as means with S.E.M. bars ($n=8$ for each group). Control rats ■, DM+ARI rats, ARI-treated diabetic rats ▨, DM rats, untreated diabetic rats □. *, $P<0.05$ vs. control rats; **, $P<0.05$ vs. DM rats.

rats (8 weeks; 7.4 ± 1.1 vs. 2.7 ± 0.6 μA/0.38 cm^2, $P<0.05$, 12 weeks; 7.0 ± 1.0 vs. 1.1 ± 0.2 μA/0.38 cm^2, $P<0.05$, each $n=8$, Fig. 2). ARI treatment alleviated dysfunction of submucosal cholinergic and peptidergic neurons. In diabetic rats with or without ARI treatment, however, CCH induced an increase in Isc in similar to that induced the control rats (8 weeks; 11.8 ± 2.0 μA/0.38 cm^2, 12 weeks; 11.9 ± 1.9 μA/0.38 cm^2).

3.4. Measurement of gastric emptying

Serum acetaminophen levels in control rats were 13.7 ± 1.3 μg/ml at 8 weeks (Fig. 3). Serum acetaminophen levels in the DM+ARI and DM rats were 7.5 ± 0.5 and 5.2 ± 0.5 μg/ml, respectively, ($P<0.05$ vs. control rats, respectively). At 8 weeks, ARI did not produce a statistically significant improvement in gastric emptying time in diabetic rats. On the other hand, the serum acetaminophen level in the DM+INS rats at 8 weeks was 9.9 ± 0.5 μg/ml and higher than those in DM rats ($P<0.05$). The serum acetaminophen level in control rats was 9.3 ± 0.8 μg/ml at 12 weeks. Serum acetaminophen levels in the DM+ARI and DM rats were 6.6 ± 0.4 and 3.5 ± 0.5 μg/ml, respectively ($P<0.05$ vs. control rats, respectively). At 12 weeks, the serum acetaminophen levels in the DM+ARI rats were significantly higher than those in the DM rats ($P<0.05$). Thus, ARI treatment alleviated the disorder in gastric emptying time, a parameter of gastric motility. The serum acetaminophen level in the DM+INS rats at 12 weeks was 7.5 ± 0.4 μg/ml and also higher than in DM rats ($P<0.05$).

4. Discussion

This is the first report to demonstrate that ARI alleviates the dysfunction of intestinal ion transport caused by diabetic autonomic neuropathy. The present study demonstrates that epalrestat inhibits the progression of disordered gastric emptying and Isc in response to EFS in diabetic rats. Changes in bowel motility and electrolyte transport have been described in both diabetes patients and animals [1,10,11].

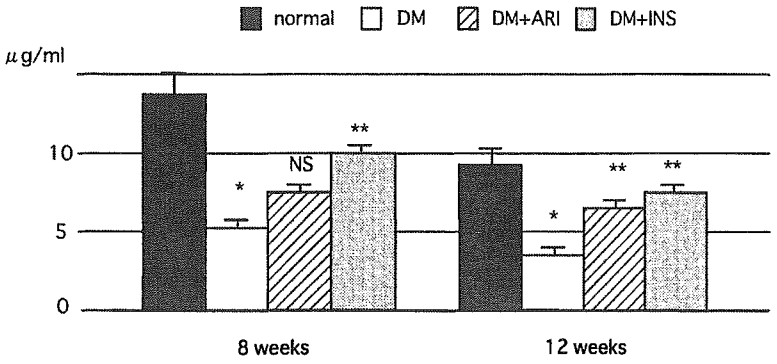

Fig. 3. Serum acetaminophen levels with or without treatment. Serum acetaminophen levels in DM+ARI and DM rats were significantly lower than in control rats at 8 and 12 weeks. At 8 weeks, serum acetaminophen levels in DM+ARI rats were similar to those in DM rats, while those in DM+INS rats were higher than those in DM rats. At 12 weeks, the serum acetaminophen levels both in DM+ARI rats and in DM+INS rats was significantly higher than in DM rats. Data are shown as means with S.E.M. bars. Experimental groups ($n = 8$): Control rats ■, DM+ARI rats, ARI-treated diabetic rats ▨, DM rats, untreated diabetic rats □, DM+INS rats, insulin-treated diabetic rats ▨. *, $P < 0.05$ vs. Control rats; **, $P < 0.05$ vs. DM rats; NS: not significant vs. DM rats.

Basal and elicited Isc appear mainly as Cl^- secretion on mucosa in rat distal colon [19]. The activation of Cl^- secretion elicited by the enteric nerve should change the gut from a state of net water absorption to one of fluid secretion. Accordingly, the decrease of Cl^- secretion due to damage of the enteric nerve causes the decrease of fluid secretion. In the current study, the STZ diabetic rats showed a defect in regulation of neuron-mediated Cl^- secretion and fluid secretion. In the ARI-treated diabetic rats, the decrease of Isc in response to EFS was significantly less than in the untreated diabetic rats.

The Isc response to EFS was abolished by the administration of TTX, suggesting mediation by neurotransmitters released from submucosal plexus neurons. However, the effect of CCH was unaffected after pretreatment of tissues with the neuronal conduction-blocker TTX, indicating that CCH acts directly on the muscarinic receptors on epithelial cells. The current study shows that EFS caused a TTX-and atropine-sensitive transient increase in Isc which was significantly less in tissues from diabetic rats than from control rats. Furthermore, the Isc response to EFS was unaffected by guanethidine, an adrenergic nerve blocker. Therefore, as found in the study by Perdue et al. [11], the reduced ion transport is more likely to have been due to abnormalities of the cholinergic nerve in the diabetic condition. In addition, in both diabetic groups, the CCH-elicited Isc response is similar to controls, suggesting that the muscarinic receptors on epithelial cells and their downstream signals are not impaired in diabetic rats.

The decreases of Cl^- and fluid secretion are related to intestinal dysfunction such as diabetic constipation. In the current study, the ratio of fecal water to fecal solids in the ARI-untreated diabetic rats was significantly decreased compared with control rats at 8 and 12 weeks after STZ treatment, showing good agreement with the ion transport study. In the vivo study, ARI also ameliorated the ratio of fecal water to fecal solids in the diabetic rats.

The Isc response to EFS in DM rats showed 75 and 90% reductions at 8 and 12 weeks, respectively, while water content in feces decreased only 15%, so there may well be other mechanisms in colonic mucosa which compensating for reduced neuron-mediated Cl^- secretion.

Constipation is the most common gastrointestinal problem in patients with diabetes mellitus, especially type 2 diabetes [20], and has been reported in as many as 20% of patients [20,21].

Although almost all of the diabetic rats in the present study had constipation, another study found that STZ treatment induced diarrhea [22]. The mechanisms of diabetic diarrhea are multifactorial and complex. Multiple pathogenic me-

chanisms such as autonomic neuropathy, bacterial overgrowth, and pancreatic exocrine function [23,24] have been implicated. In addition, the deterioration of different types of nerves might occur at different rates under different circumstances to produce contrary consequences of diarrhea and constipation.

Several highly sensitive methods are available to assess bowel motor function in vivo. The acetaminophen method for gastric emptying time is versatile and reliable [13]. We administered 20 mg of acetaminophen to each group of rats, regardless of body weight. As 12-week control rats weighed more than 8-week control rats, a lesser serum acetaminophen level was expected. However, DM rats and DM+ARI rats at 12 weeks had similar body weight, as shown in Table 1. In DM rats and DM+ARI rats at 12 weeks, therefore, the difference of serum acetaminophen level should reflect gastric emptying.

Recent reports indicate that acute hyperglycemia causes reversible impairment of motility in various regions of the gastrointestinal tract [25,26]. In the present study, there were no differences in blood glucose levels between untreated and ARI-treated diabetic rats throughout the recording period, but ARI treatment inhibited progression of disordered gastric motility in the 12-week-diabetic rats. Accordingly, metabolic disorders of exaggerated flux through the polyol pathway may play a more important role in diabetic gastroparesis than hyperglycemia itself. In 8-week-diabetic rats, on the other hand, ARI produced an insignificant improvement of gastric emptying, so the delayed gastric emptying observed in 8-week-diabetic rats also should be due mainly to neuropathy. However, we are not able to exclude a role of hyperglycemia itself in the delayed gastric emptying observed in 8-weeks-diabetic rats.

From a clinical point of view, symptoms of nausea and vomiting frequently manifest delayed gastric emptying in diabetic patients. In some patients, these symptoms can limit oral nutrition and contribute to morbidity. Furthermore, gastroparesis contributes to poor glycemic control due to unpredictable oral intake as well as to poor absorption of nutrients due to delayed gastric emptying. Treatment of gastroparesis is important to attain fair glycemic control. The major treatments for diabetic gastroparesis are dietary adjustments and the use of gastric prokinetic agents [27,28]. ARI treatment also may have a beneficial effect in the management of diabetic gastroparesis.

Kikkawa et al. [29] reported that epalrestat ameliorated peripheral nerve disorder in STZ-induced diabetic rats by reducing the sorbitol content in the sciatic nerve, a somatic nerve, in a dose-dependent manner. Sima et al. have reported regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy who were treated with sorbinil [30]. ARI may similarly inhibit intracellular excess of sorbitol, at least in intrinsic neuron of the autonomic nerve in the gastrointestinal tract.

Several investigators have reported the efficacy of insulin therapy on diabetic neuropathy. Yoshida et al. found that insulin therapy prevented the dysfunction of sympathetic nerve activity in STZ diabetic rats [31]. Nowak et al. found that insulin therapy improved the abnormality in cholinergic neuromuscular transmission in small intestine in STZ diabetic rats [32]. In the present study, insulin therapy inhibited the progression of disordered gastric emptying in STZ diabetic rats, showing good agreement with their study.

Yoshida et al. reported that ARI treatment in control rats has no effect on norepinephrine turnover, motor nerve conduction velocity, and sorbitol content [31], indicating the effects of ARI found in the present study are relevant to diabetes therapy.

Over the past 20 years, results of ARI trials have not demonstrated its efficacy [33]. One of the reasons is that the stage of the neuropathy has not been considered appropriately. As Pfeifer et al. hypothesized, abnormal nerve function can be divided conceptually into a metabolic component, which theoretically can be rapidly reversed and is greatest in the early stages of neuropathy, and a structural component, which occurs later in the disease process and is more permanent [6]. Accordingly, it is thought that starting treatment in the early stages of diabetic gastroenteropathy is the key to success.

In conclusion, our findings indicate that ARI treatment ameliorates gastrointestinal physiological dysfunction by preventing enteric nerve disorder in diabetic rats, and suggest that it may be useful in early treatment of diabetic gastroenteropathy.

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Factors Responsible for Development From Normal Glucose Tolerance to Isolated Postchallenge Hyperglycemia

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OBJECTIVE — Isolated postchallenge hyperglycemia (IPH), defined as fasting plasma glucose (FPG) level <7.0 mmol/l and 2-h plasma glucose (PG) level ≥11.1 mmol/l, is a subtype of early-stage diabetes. This study evaluates the metabolic profiles of insulin secretion and insulin sensitivity in IPH to clarify the factors responsible for development of this form of type 2 diabetes.

RESEARCH DESIGN AND METHODS — We conducted cross-sectional analysis of 231 Japanese men aged 20–70 years. The subjects were classified into the following three groups, based on the results of a 75-g oral glucose tolerance test (OGTT): *1*) normal glucose tolerance (NGT), defined as FPG level <6.1 mmol/l and 2-h PG level <7.8 mmol/l (n = 89); 2) impaired glucose tolerance (IGT), defined as FPG level <7.0 mmol/l and 2-h PG level of 7.8–11.1 mmol/l (n = 94); and 3) IPH (n = 48). We compared the three groups for insulin secretion (insulinogenic index) and insulin sensitivity (index of insulin resistance using homeostasis model assessment [HOMA-IR]).

RESULTS — The insulinogenic index in IPH was the lowest of the three groups ($P < 0.001$ versus NGT). The HOMA-IR in the IGT and IPH groups were significantly higher than in the NGT group ($P < 0.001$), but both were similar. By linear regression analysis, the insulinogenic index rather than fasting insulin or HOMA-IR was the more significant factor in the 2-h PG level in IGT and IPH.

CONCLUSIONS — Subjects with IPH exhibited distinctly impaired early-phase insulin secretion and only mild insulin resistance, indicating that reduced insulin secretion is the primary determinant of deterioration from NGT to IGT and IPH in development of type 2 diabetes in these subjects.

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Postprandial hyperglycemia is recognized as an important risk factor for diabetic complications. According to long-term follow-up data from the Honolulu Heart Program and the Diabetes Intervention Study (1,2), postprandial hyperglycemia is a significant predictor of myocardial infarction and mortality. Postprandial hyperglycemia itself also may play an important role in the development of type 2 diabetes, reducing the secretory response of β-cells and impairing insulin-mediated glucose transport, exacerbating metabolic deterioration (3,4).

Postchallenge hyperglycemia after oral glucose tolerance test (OGTT) is an appropriate model of postprandial hyperglycemia. In previous studies (5–7), subjects with isolated postchallenge hyperglycemia (IPH) have been shown to have higher risk of cardiovascular disease and mortality. In addition, some studies have suggested that postchallenge hyperglycemia represents a phenotype of early-stage overt diabetes (8).

The metabolic characteristics of IPH are of clinical importance for the prevention of diabetic complications and for early intervention. In this cross-sectional study, we compared insulin secretion and insulin sensitivity in subjects with IPH, impaired glucose tolerance (IGT), and normal glucose tolerance (NGT) to determine the metabolic characteristics of IPH in Japanese men and to evaluate the factors responsible for the deterioration of postload glucose regulation in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects

We recruited 379 consecutive Japanese men undergoing 75-g OGTT for closer evaluation because of family history of diabetes, positive result of urine glucose test, or HbA_{1c} level >5.0% at the initial examination for regular medical checkup, at Kyoto University Hospital, Ikeda Hospital, Kansai-Denryoku Hospital, and Kansai Health Management Center between 1991 and 2001. In all subjects, OGTT was performed within 3 months of the initial examination. All subjects were Japanese men aged 20–70 years who showed no signs of hypertension or hepatic, renal, or endocrine diseases, engaged in no heavy exercise, and were not taking any medications before the study. The study was designed in compliance

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Abbreviations: FPG, fasting plasma glucose; HOMA-IR, index of insulin resistance using homeostasis model assessment; IGT, impaired glucose tolerance; IGT/FH, IGT with fasting hyperglycemia; IPH, isolated postchallenge hyperglycemia; ISI, insulin sensitivity index; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PG, plasma glucose.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Demographic/metabolic characteristics of the NGT, IGT, and IPH groups*

	NGT	IGT	IPH	Total
n	89	94	48	231
Age (years)	45.6 ± 1.1	52.9 ± 0.9‡	52.0 ± 1.0‡	49.9 ± 0.6
BMI (kg/m^2)	23.9 ± 0.3	24.1 ± 0.3	24.7 ± 0.5	24.2 ± 0.2
Systolic blood pressure (mmHg)	128 ± 2	128 ± 2	131 ± 4	128 ± 1
Diastolic blood pressure (mmHg)	78 ± 2	77 ± 1	78 ± 2	78 ± 1
FPG (mmol/l)	5.3 ± 0.0	6.0 ± 0.1‡	6.4 ± 0.1‡	5.8 ± 0.0
2-h PG (mmol/l)	5.8 ± 0.1	9.3 ± 0.1	13.0 ± 0.2‡	8.7 ± 0.2
Fasting insulin (pmol/l)	27 ± 1	38 ± 2†	37 ± 4†	33 ± 1
2-h insulin (pmol/l)	168 ± 15	259 ± 11‡	235 ± 28†	219 ± 11
HbA_{1c} (%)	5.5 ± 0.1	5.9 ± 0.1‡	6.4 ± 0.1‡	5.9 ± 0.0
Triglycerides (mmol/l)	1.28 ± 0.09	1.97 ± 0.24†	2.22 ± 0.22†	1.73 ± 0.11
Total cholesterol (mmol/l)	5.09 ± 0.10	5.26 ± 0.10*	5.45 ± 0.11	5.24 ± 0.06
HDL cholesterol (mmol/l)	1.28 ± 0.04	1.12 ± 0.03†	1.20 ± 0.05	1.20 ± 0.05

Data are means ± SE. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ versus NGT.

with the ethics regulations set out by the Helsinki Declaration.

Each standard OGTT was administered according to the National Diabetes Data Group recommendations (9), which require the subjects to fast overnight for 10–16 h. Blood samples for determination of blood glucose levels were collected 0, 30, 60, 90, and 120 min after oral administration of 75 g glucose. Blood samples for measurement of HbA_{1c}, insulin, total cholesterol, HDL cholesterol, and triglyceride levels were collected after an overnight fast.

Because our focus is on postload hyperglycemia, we selected subjects with NGT, IGT, and IPH from the original sample (n = 379). Definition of NGT and IGT were according to the 1998 World Health Organization (WHO) diagnostic criteria (10). The NGT group comprised the subjects with fasting plasma glucose (FPG) level <6.1 mmol/l and 2-h plasma glucose (PG) <7.8 mmol/l (n = 89). The IGT group comprised the subjects with FPG level <7.0 mmol/l and 2-h PG level of 7.8–11.1 mmol/l (n = 94). Subjects with FPG level <7.0 mmol/l and 2-h PG level ≥11.1 mmol/l comprised the IPH group (n = 48). The definition of IPH in the present study is consistent with recent large epidemiologic studies (6–8). The mean BMI of the subjects enrolled in this study (n = 231) is similar to that in Japanese diabetic or IGT subjects in a population-based study: IGT 24.1 vs. 24.4 kg/m^2 and diabetes (IPH) 24.7 vs. 24.3 kg/m^2, respectively (11).

For further analysis, the IGT group was divided into isolated IGT, defined as FPG <6.1 mmol/l (n = 48), and IGT with fasting hyperglycemia (IGT/FH), defined as FPG 6.1–7.0 mmol/l (n = 46), because recent studies have shown that the metabolic characteristics of subjects with IGT/FH are more deteriorated than in isolated IGT (12,13).

Measurements

PG was measured by glucose oxidase method using the Hitachi Automatic Clinical Analyzer 7170 (Hitachi, Tokyo, Japan). Serum insulin was measured by radioimmunoassay (Dainabot, Tokyo, Japan). Serum total cholesterol, HDL cholesterol, and triglyceride levels were measured as reported previously (14).

As the index of insulin secretion, we used the insulinogenic index, the change in the ratio of insulin to glucose level during the first 30 min of OGTT (30-min insulin level − fasting insulin)/(30-min PG − FPG) (15,16). As the measure of insulin resistance, we used the index of insulin resistance by homeostasis model assessment (HOMA-IR) calculated by the formula of FPG (mmol/l) × fasting insulin (mU/l)/22.5 (17). HOMA-IR is a reasonable measure of insulin resistance, correlating well with values obtained by glucose clamp and minimal model studies (18,19).

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences version 10.0J (SPSS, Chicago, IL). Age, BMI, systolic and diastolic blood pressure, FPG/2-h PG, HbA_{1c}, fasting insulin, triglycerides, total cholesterol, HDL cholesterol, insulinogenic index, and HOMA-IR were compared among the NGT, IGT, and IPH groups by general ANOVA. For comparison with NGT, unpaired Student's t test was performed as post hoc analysis. When the IGT group was divided into isolated IGT and IGT/FH, comparisons of the metabolic profiles among isolated IGT, IGT/FH, and IPH were performed using ANOVA. Linear regression analysis was performed in the IGT and IPH subjects with fasting and 2-h PG measurements as dependent variables and fasting insulin level, HOMA-IR, and the insulinogenic index as independent variables. P values <0.05 were considered statistically significant. Data are expressed as means ± SE.

RESULTS — The clinical and metabolic characteristics of the subjects are shown in Table 1. A total of 231 subjects were enrolled in the study. The average (± SE) age and BMI were 49.9 ± 0.6 years and 24.2 ± 0.2 kg/m^2, respectively. There was no significant difference in BMI and blood pressure among the groups. The mean age of the NGT group was significantly younger than the others ($P <$ 0.001), but there was no significant difference in age between the IGT and IPH groups. The fasting insulin level in the IPH group was significantly higher than in the NGT group ($P < 0.01$) but similar to that in the IGT group. On the other hand, the 30-min insulin level was significantly lower in the IPH group than in the NGT group (199 and 159 pmol/l, respectively; $P < 0.001$). In the IPH group, the 2-h insulin level was significantly higher

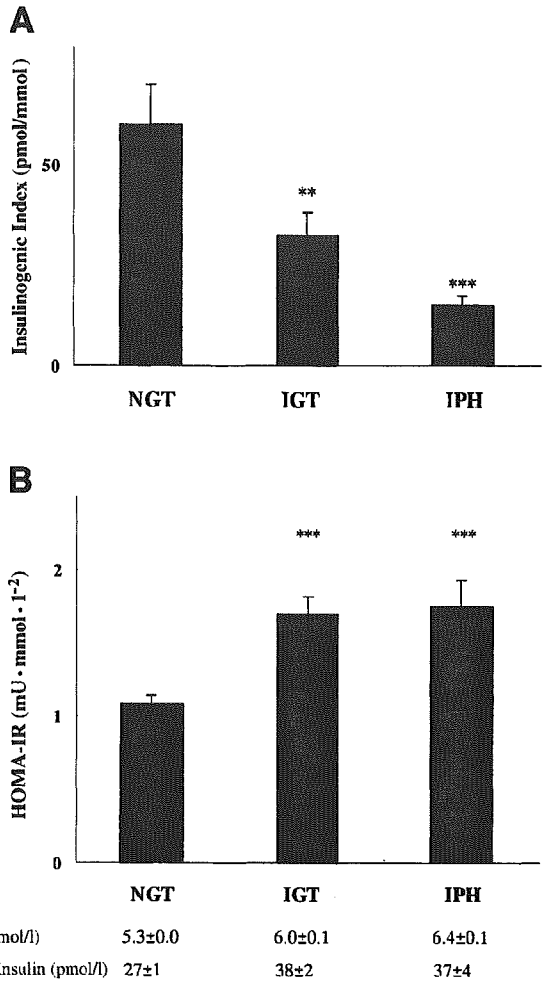

Figure 1—A: *Insulinogenic index in subjects with NGT, IGT, and IPH. Data are means ± SE.* **P < *0.01;* ***P < *0.001 versus NGT.* B: *HOMA-IR in subjects with NGT, IGT, and IPH. Data are means ± SE.* ***P < *0.001 versus NGT.*

than in the NGT group (235 and 168 pmol/l, respectively; $P < 0.05$) and marginally lower than in the IGT group (259 pmol/l; $P = 0.07$), although the IPH group had a higher 2-h PG level than the NGT and IGT groups. The IPH group had a significantly higher triglyceride level than the NGT group ($P = 0.001$), but there was no significant difference in total cholesterol or HDL cholesterol levels between the NGT and IPH groups.

Comparisons of the insulinogenic index among the groups are shown in Fig. 1*A*. The insulinogenic index in the IPH group was lower than the others ($P < 0.001$ versus NGT). Comparisons of HOMA-IR among the groups are shown in Fig. 1*B*. HOMA-IR in the IGT and IPH groups were similar, but both were significantly higher than the NGT group ($P < 0.001$).

Metabolic characteristics and comparisons of the isolated IGT, IGT/FH, and IPH groups are shown in Table 2. The insulinogenic index in the IPH group was significantly lower than the isolated IGT group ($P < 0.05$), but there was no significant difference between the isolated IGT and IGT/FH groups. On the other hand, HOMA-IR in the IGT/FH group was significantly higher than in the isolated IGT group ($P < 0.01$), but there was no significant difference between the IPH and isolated IGT groups.

Linear regression analysis of both FPG and 2-h PG levels and the fasting insulin, HOMA-IR, and insulinogenic index in IGT and IPH subjects is shown in Table 3. FPG correlated significantly with fasting insulin as a measure of insulin resistance ($P = 0.004$) but not with the insulinogenic index ($P = 0.074$). On the other hand, 2-h PG correlated well with the insulinogenic index ($P = 0.030$) but not with fasting insulin or HOMA-IR ($P = 0.396$ and 0.162, respectively).

CONCLUSIONS— In the present study, we have clarified the metabolic profile of IPH regarding insulin secretion and insulin resistance. Both insulin secretion and insulin action in IPH subjects are reduced significantly compared with NGT subjects. However, in contrast to similar insulin resistance, IPH subjects exhibit considerably less insulin secretion than IGT subjects. Moreover, when IGT is separated into isolated IGT and IGT/FH, IPH subjects show significantly lower insulin secretion than isolated IGT subjects,

Table 2—*Metabolic comparisons among the isolated IGT, IGT/FH, and IPH groups*

	Isolated IGT	IGT/FH	IPH
n	48	46	48
Age (years)	51 ± 1.3	55 ± 1.3*	52 ± 1.0
BMI (kg/m^2)	23.6 ± 0.4	24.4 ± 0.4	24.7 ± 0.5
Systolc blood pressure (mmHg)	124 ± 3	131 ± 3	131 ± 4
Diastolic blood pressure (mmHg)	75 ± 2	79 ± 2	78 ± 2
FPG (mmol/l)	5.6 ± 0.0	6.4 ± 0.0‡	6.4 ± 0.1‡
2-h PG (mmol/l)	9.0 ± 0.1	9.5 ± 0.1	13.0 ± 0.2‡
Fasting insulin (pmol/l)	32 ± 2	40 ± 4*	37 ± 4
HbA_{1c} (%)	5.5 ± 0.1	6.0 ± 0.1	6.4 ± 0.1‡
Triglycerides (mmol/l)	2.24 ± 0.41	1.61 ± 0.13	2.22 ± 0.22
Total cholesterol (mmol/l)	5.21 ± 0.15	5.33 ± 0.14	5.45 ± 0.11
HDL cholesterol (mmol/l)	1.07 ± 0.05	1.19 ± 0.05	1.20 ± 0.05
Insulinogenic index (pmol · mmol^{-1})	34 ± 8	30 ± 8	15 ± 2*
HOMA-IR (mU · mmol · l^{-2})	1.3 ± 0.1	1.9 ± 0.2†	1.8 ± 0.2

Data are mean ± SE. *$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$ versus isolated IGT.

Table 3—*Linear correlation analysis of FPG and 2-h PG levels with fasting insulin, HOMA-IR, and insulinogenic index in IGT and IPH subjects (n = 142)*

		Fasting insulin	HOMA-IR	Insulinogenic index
FPG	*r*	0.239	—	−0.160
	P	0.004	—	0.074
2-h PG	*r*	0.072	0.118	−0.195
	P	0.396	0.162	0.030

whereas insulin resistance is not significantly different. These results suggest that progression from NGT via IGT or isolated IGT to IPH in these subjects is due mostly to deterioration of early-phase insulin secretion and to a lesser contribution of insulin resistance.

For further clarification of the metabolic characteristics of IPH, we compared the insulinogenic index and HOMA-IR of IPH subjects with newly diagnosed overt diabetes from our original sample (FPG ≥7.0 mmol/l; *n* = 118). Subjects with IPH had higher insulinogenic index and lower HOMA-IR than those with overt diabetes (11.2 vs. 8.6 pmol · $mmol^{-1}$ and 1.76 vs. 2.19 mU · mmol · l^{-2}, respectively). These results suggest that IPH is a less metabolically deteriorated status than overt diabetes.

According to DeFronzo et al. (20), progression from NGT to type 2 diabetes with FPG <7.8 mmol/l correlates well with increased insulin resistance but less well with impairment of early-phase insulin secretion. The decline in β-cell function becomes apparent only after FPG exceeds 7.8 mmol/l. Gerich (21) has investigated insulin resistance in the prediabetic state and suggests the importance of impaired insulin secretion in progression to diabetes from NGT. The importance of insulin resistance at early stages of diabetes has been shown mostly in studies of obese subjects (22–24), who have higher insulin resistance. After excluding the influence of obesity in previous analyses, Gerich (25) showed that insulin resistance is frequently not the primary factor in the development of type 2 diabetes. The present study clearly shows, in nonobese Japanese diabetic subjects, that deterioration of β-cell function plays a very important role in progression from IGT to IPH.

Although HOMA-IR is a common index for evaluating insulin sensitivity and correlates well with both hepatic sensitivity measured by glucose tracer method and whole-body insulin sensitivity measured by glucose clamp method (17), it is believed to reflect hepatic insulin sensitivity more than peripheral insulin sensitivity (26). We also analyzed the insulin sensitivity of our study subjects by the insulin sensitivity index (ISI) composite proposed by Matsuda and DeFronzo (26), which is considered a more accurate measurement of systemic insulin resistance. The ISI composite is obtained from OGTT results and calculated by the formula of 10,000/square root (FPG × fasting insulin × mean OGTT glucose concentration × mean OGTT insulin concentration). There was no difference in insulin resistance between the IGT and IPH groups by this measurement (means ± SE were 7.3 ± 0.5 and 6.8 ± 0.6, respectively; $P = 0.557$).

In addition to the metabolic characteristics of IPH, the present study shows the metabolic characteristics of IGT in presence of fasting hyperglycemia. In subjects with IGT/FH, fasting insulin level and HOMA-IR were higher than in subjects with isolated IGT, whereas the insulinogenic index in subjects with IGT/FH was slightly lower than or similar to isolated IGT. These results indicate that deterioration from isolated IGT to IGT/FH is due to insulin resistance, in contrast to the deterioration to IPH. Linear regression analysis in IGT and IPH subjects also supports the theory that postload hyperglycemia is caused mostly by deterioration of insulin secretion and fasting hyperglycemia is caused by insulin resistance. Although the causes of fasting and 2-h hyperglycemia are controversial (12,13, 20,25), O'Rahilly et al. (23) and others have found that early insulin secretion is more closely related to FPG than to postload glucose level, whereas fasting hyperglycemia depends more on insulin resistance (13). The discrepancy may be due to the focus of the present study on subjects with IPH rather than those with IGT or diabetes. Ethnic and other population differences also may be a factor (27). The subjects of the present study, nonobese, middle-aged, Japanese men with early-stage type 2 diabetes, are more homogeneous than those of other studies, and other studies with lean and/or Japanese subjects also have shown that insulin secretion is an important factor in the development of postload hyperglycemia (28,29).

The present study is limited in that no insight is provided into the time course of the development of these abnormalities in insulin secretion and action. However, IPH clearly is characterized by considerably impaired early insulin secretion and mild insulin resistance, indicating that deterioration of insulin secretion is a strong determinant of progression from NGT via IGT or isolated IGT to IPH in this study population. However, insulin resistance is the stronger determinant of deterioration from isolated IGT to IGT/FH. The present study also indicates that results of 75-g OGTT may be clinically useful to classify patients for establishment of appropriate prevention and treatment in early-stage type 2 diabetes.

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Platelet Count Is Independently Associated With Insulin Resistance in Non-obese Japanese Type 2 Diabetic Patients

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The aim of the present study was to investigate the relationship between platelet count and insulin resistance in non-obese Japanese type 2 diabetic patients. A total of 163 non-obese Japanese type 2 diabetic patients (112 men and 51 women, aged 36 to 84 years, body mass index [BMI] 16.2 to 26.9 kg/m^2) were studied. In conjunction with BMI, glycosylated hemoglobin (HbA_{1c}), fasting concentrations of plasma glucose and serum lipids (triglycerides, low-density lipoprotein [LDL] cholesterol, high-density lipoprotein [HDL] cholesterol, and total cholesterol), and hematological parameters (platelets, white blood cell count, red blood cell count, hematocrit, hemoglobin) were measured. LDL cholesterol was calculated using the Friedewald formula. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment (HOMA-IR). Univariate regression analysis showed that HOMA-IR was positively correlated to BMI ($r = 0.465$, $P < .0001$), HbA_{1c} ($r = 0.423$, $P < .0001$), platelet count ($r = 0.310$, $P < .0001$), triglycerides ($r = 0.277$, $P < .0005$), white blood cell count ($r = .222$, $P = .005$), red blood cell count ($r = 0.210$, $P = .008$), hematocrit ($r = 0.156$, $P = .047$), total cholesterol ($r = 0.178$, $P = .023$), and systolic ($r = 0.216$, $P = .011$) and diastolic ($r = 0.263$, $P = .002$) blood pressure, and inversely correlated to HDL cholesterol ($r = -0.312$, $P < .0001$) level in our diabetic patients. Multiple regression analysis showed that HOMA-IR was independently predicted by BMI ($P < .0001$, $F = 22.45$), HbA_{1c} ($P < .0001$, $F = 16.15$), platelet count ($P < .0001$, $F = 10.75$), and serum triglycerides ($P < .0001$, $F = 10.47$) levels, which explained 34% of the variability of HOMA-IR in non-obese Japanese type 2 diabetic patients. These results indicate that not only BMI, HbA_{1c}, and triglycerides levels but also platelet counts are independent predictor of insulin resistance in non-obese Japanese type 2 diabetic patients.

THE MAJOR clinical consequence of type 2 diabetes is mortality and morbidity from atherosclerotic vascular disease. Bierman[1] has previously estimated that typical risk factors, including smoking, cholesterol level, and blood pressure, can account for no more than 25% to 30% of excess cardiovascular risk factor in diabetic patients. This suggests that other factors might play a key role in the progression of atherosclerosis in diabetes.

Insulin resistance is established to be one of the risk factors for the coronary heart disease events.[2] There are some data suggesting that hematologic abnormalities are associated with atherosclerosis in humans.[3-6] High red blood cell count is known to be a strong independent predictor of acute cardiovascular events such as stroke and myocardial infarction. Endothelial injury or plaque rupture with platelet adhesion and aggregation at the site of injury may be the crucial event in producing morbidity and mortality from atherosclerosis.

To the best of our knowledge, the relationship between hematological parameters and the degree of insulin resistance has not been fully clarified in type 2 diabetic patients. One problem is that the degree of weight excess and of hyperglycemia per se effects hematological parameters and insulin resistance in man. To overcome this difficulty, we recruited non-obese well-controlled unique Japanese type 2 diabetic patients who had no evidence of cardiovascular disease, ischemic stroke, or chronic renal failure and investigated the relationships between hematologic parameters and the degree of insulin resistance.

SUBJECTS AND METHODS

One hundred sixty-three non-obese Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. Type 2 diabetes mellitus was diagnosed based on the World Health Organization (WHO) criteria.[7] The subjects had no evidence of current acute illness or infectious process. The duration of diabetes was 11.6 ± 0.7 years (mean ± SEM). One hundred eight of 163 diabetic patients were taking sulfonylureas (gliclazide) and the rest were controlled with diet alone. None had received insulin therapy. All subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease. They did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with glucose oxidase method. Triglycerides, and total and high-density lipoprotein (HDL) cholesterol were measured. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.[8] Platelets, white blood cell count, red blood cell count, hematocrit, and hemoglobin were also measured.

The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/L)/22.5.[9] One might argue that the use of sulfonylureas in patients with diabetes might significantly affect the estimate of insulin resistance by HOMA, as these drugs are known to decrease fasting plasma glucose without substantially changing fasting plasma insulin.[10] Bonora et al[11] and Emoto et al,[12] however, confirmed that in the validation studies of HOMA, the

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Table 1. Clinical Characteristics in Insulin-Resistant and Insulin-Sensitive Diabetic Patients

	Insulin-Resistant	Insulin-Sensitive	*P*
No. of subjects	66	97	
Male/female	46/20	66/31	.412
HOMA-IR	3.9 ± 0.2	1.6 ± 0.1	<.001
Age (yr)	60.4 ± 1.2	61.6 ± 1.0	.214
Diabetes duration (yr)	12.1 ± 1.1	11.2 ± 0.8	.299
BMI (kg/m^2)	23.7 ± 0.2	21.8 ± 0.3	<.001
HbA_{1c} (%)	7.6 ± 0.1	6.7 ± 0.1	<.001
Fasting glucose (mg/dL)	162 ± 4	132 ± 3	<.001
Fasting insulin (μU/mL)	9.7 ± 0.5	4.9 ± 0.2	<.001
Triglycerides (mg/dL)	164 ± 28	110 ± 6	.013
Total cholesterol (mg/dL)	210 ± 4	195 ± 4	.005
LDL cholesterol (mg/dL)	129 ± 6	115 ± 3	.013
HDL cholesterol (mg/dL)	48 ± 1	58 ± 2	<.001
Red blood cell count (× 10^4/μL)	453 ± 5	438 ± 5	.016
White blood cell count (/μL)	5872 ± 154	5511 ± 140	.047
Platelets (× 10^4/μL)	22.3 ± 0.6	18.8 ± 0.5	<.001
Hemoglobin (g/dL)	14.0 ± 0.1	13.8 ± 0.2	.124
Hematocrit (%)	41.5 ± 0.4	40.8 ± 0.4	.134

correlation of insulin sensitivity estimated by such method and that measured by the glucose clamp was not substantially different in diet-treated and sulfonylurea-treated type 2 diabetes.

Statistical Analysis

Data were presented as means ± SEM. Statistical analyses were conducted using the StatView 5 system (Statview, Berkeley, CA). Simple (Spearman's rank) correlation coefficient and stepwise multiple regression analyses were used to examine the relationships between HOMA-IR and body mass index (BMI), triglycerides, or the measures of variables including platelet count. $P < .05$ was considered as significant. In multivariate analysis, an F value ≥ 4 was considered significant.

RESULTS

The subjects studied were all Japanese type 2 diabetic patients (112 men and 51 women) with an age range of 36 to 84 years (61.1 ± 0.8 years) and a BMI of 16.2 to 26.9 kg/m^2 (22.6 ± 0.2 kg.m^2). They all were non-obese.[13] The mean fasting plasma glucose was 144 ± 3 mg/dL and glycosylated hemoglobin (HbA_{1c}) was 7.1% ± 0.1%. Fasting insulin level was 6.8 ± 0.3 μU/mL. Serum triglycerides, and total and HDL cholesterol levels were 132 ± 12 mg/dL, 201 ± 3 mg/dL, and 54 ± 1 mg/dL, respectively. Platelet, white blood cell, and red blood cell counts were 20.2 ± 0.4 × 10^4/μL, 5,658 ± 107/μL, and 444 ± 3 × 10^4/μL, respectively. Hematocrit and hemoglobin concentrations were 41.1% ± 0.3% and 13.9 ± 0.1 g/dL, respectively. There was a wide variation in insulin resistance calculated from HOMA-IR in our diabetic patients (range, 0.54 to 12.74; 2.49 ± 0.13). Sixty-six of 163 (40%) patients had HOMA-IR greater than 2.5, indicating they were insulin-resistant.[14]

Table 1 shows the clinical profile of insulin-resistant and insulin-sensitive type 2 diabetic patients. Compared with insulin-sensitive patients, insulin-resistant patients had significantly higher BMI levels and of HbA_{1c}, triglycerides, total and LDL cholesterol, red blood cell count, white blood cell count, and platelets. HDL cholesterol concentration was significantly lower in insulin-resistant group than in insulin-sensitive group. No significant difference was observed in age, gender, hemoglobin, or hematocrit levels between the 2 groups.

Table 2 illustrates the correlation between HOMA-IR and BMI or the measures of variables including platelet count and serum triglycerides in our diabetic patients. HOMA-IR was positively correlated with BMI ($r = 0.465$, $P < .0001$), hemoglobin A_{1c} ($r = 0.423$, $P < .0001$), platelets ($r = 0.310$, $P < .0001$), triglycerides ($r = 0.277$, $P < .0005$), total ($r = 0.178$, $P = .023$) and LDL ($r = 0.250$, $P = .002$) cholesterol, white blood cell count ($r = 0.222$, $P = .005$), red blood cell count ($r = 0.210$, $P = .008$), hematocrit ($r = 0.156$, $P = .047$), and systolic ($r = 0.216$, $P = .011$) and diastolic ($r = 0.263$, $P = .002$) blood pressure. In contrast, HOMA-IR value was negatively correlated with HDL cholesterol level ($r = -0.312$, $P < .0001$). There was no relationship between HOMA-IR and age, gender, duration of diabetes, or hemoglobin.

Multiple regression analyses were performed using the stepwise procedure in all diabetic patients. The analysis included HOMA-IR as dependent variable and candidate risk factors (BMI, HbA_{1c}, plateleta, triglycerides, total, LDL, and HDL cholesterol, white blood cell count, red blood cell count, hematocrit, systolic and diastolic blood pressure) as independent variables. HOMA-IR was statistically predicted by BMI ($P < .0001$, $F = 22.45$), HbA_{1c} ($P < 0.0001$, $F = 16.15$), platelet count ($P < .0001$, $F = 10.75$), and serum triglycerides ($P < .0001$, $F = 10.47$) levels, which explained 34% of the variability of HOMA-IR in our diabetic patients. Other variables, including red blood cell count, were not associated with HOMA-IR in our non-obese Japanese type 2 diabetic patients (Table 2).

DISCUSSION

Our study demonstrated that platelet counts are a statistically predictor of insulin resistance in non-obese Japanese type 2 diabetic patients. Our patients studied were unique in that they

Table 2. Correlation of Insulin Resistance to Measures of Variables in Diabetic Patients

	Univariate		
	r	*P*	Multivariate *F*
BMI	0.465	<.0001	22.45
HbA_{1c}	0.423	<.0001	16.15
Platelets	0.310	<.0001	10.75
Triglycerides	0.277	<.0005	10.47
Total cholesterol	0.178	.023	—
LDL cholesterol	0.250	.002	—
White blood cell count	0.222	.005	—
Red blood cell count	0.210	.008	—
Hematocrit	0.156	.047	—
Systolic blood pressure	0.216	.011	—
Diastolic blood pressure	0.263	.002	—
HDL cholesterol	−0.312	<.0001	—
Age	−0.137	.082	—
Gender	−0.006	.940	—
Diabetes duration	0.049	.583	—
Hemoglobin	0.144	.066	—

were non-obese well-controlled in terms of glucose tolerance (mean HbA_{1c}, 7.1%) and blood pressure (mean, 129/75 mm Hg). They all have received neither insulin therapy nor any medications affecting platelet function such as aspirin and dipyridamole.

When compared with Caucasian populations, non-obese Japanese type 2 diabetic patients are unique in that they are divided into 2 variants: one with insulin resistance and the other with normal insulin sensitivity.[15,16] In the present study, only 40% of the patients had insulin resistance by homeostasis model assessment, consistent with our previous study.[17] The patients with insulin resistance are characterized by higher concentrations of C-reactive protein (CRP), triglycerides, and lower concentrations of HDL cholesterol as compared to the non–insulin-resistant group.[14,17,18] Both CRP and dyslipidemia are postulated to be atherogenic. Thus, insulin resistance seems to be associated with atherosclerosis in non-obese Japanese type 2 diabetic patients.

There are some data suggesting that hematologic parameters are associated with atherosclerosis in humans.[3-6] High red blood cell counts are known to be a strong independent predictor of acute cardiovascular events such as stroke and myocardial infarction. Endothelial injury or plaque rupture with platelet adhesion and aggregation at the site of injury may be the crucial event in producing morbidity and mortality from atherosclerosis. To the best of our knowledge, however, the relationship between hematologic parameters and insulin resistance has not yet been fully investigated in type 2 diabetic patients.

By univariate analysis, we found a positive correlation among platelet, red blood cell, and white blood cell counts and insulin resistance; by multivariate analysis, we found that both red blood cell and white blood cell counts are no longer independent factors of insulin resistance. Thus, platelet count per se seems to be associated with insulin resistance in non-obese Japanese type 2 diabetic patients. This finding is not in agreement with the data shown by Barbieri et al[4] that increased red blood cell count is an aspect of insulin resistance syndrome in nondiabetic subjects. The reason for the discrepancy is currently unknown, but it may be due to the different clinical characteristics studied. Our present finding is very interesting since platelets retain a functional insulin receptor capable of insulin binding and autophosphorylation and since insulin is thought to reduce platelet sensitivity to aggregating agents such as adenosine diphosphate (ADP).[19-21]

The mechanism by which increased platelet count is associated with insulin resistance in non-obese Japanese type 2 diabetic patients is not known at present. This relationship has not been reported in nondiabetic subjects.[4] Compared with nondiabetic subjects, diabetic patients are known to have both endothelial damage and platelet dysfunction.[6]

In conclusion, we first showed in vivo a relationship between platelet count and insulin resistance in non-obese Japanese type 2 diabetic patients. Further study should determine whether improvement in insulin resistance in this population is associated with a decreased platelet count.

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