THE TWO main causes of hyperglycemia in type 2 diabetes mellitus are impaired insulin secretion and increased insulin resistance [1, 2]. Evaluation of insulin resistance (or sensitivity) and β-cell function is important for understanding the disease status and selection of pharmacologic treatment. The gold standard of evaluation of insulin sensitivity is glucose clamp test [3]. However, the test is limited to research use and is difficult to perform at every medical institution. Although there are also other tests, they are often complex or inadequate [4, 5]. Homeostasis model assessment, first described by Matthews et al., is a method for estimating insulin sensitivity [6]. This model is based on the theory of a feedback loop between β cells and the liver [7]. The homeostasis model assessment of insulin resistance (HOMA-IR), calculated from fasting plasma glucose level and immunoreactive insulin (IRI), is a simple method for evaluating insulin sensitivity and correlates with the results of glucose clamp test in subjects with mild diabetes without significant hyperglycemia [8]. Nevertheless it is difficult to apply to patients with poor glycemic control [9], those with severe β cell dysfunction [10] or those treated with insulin.

Chronic hyperglycemia is known to induce insulin secretion defect and worsen insulin resistance [11]. This phenomenon, called glucotoxicity, is partly reversible in insulin resistance, IRI: immunoreactive insulin, BMI: body mass index, FPG: fasting plasma glucose, BMI: body mass index, eVFA: estimated visceral fat area, CPR: C-reactive protein, ΔCPR: increment of CPR with the glucagon stimulation test, M/I values: insulin sensitivity index estimated with the clamp test, KITT: insulin sensitivity index estimated with the insulin tolerance test, I.I.: insulinogenic index.
Glycemic control is required before evaluation of insulin sensitivity in patients with poor glycemic control. In this regard, insulin sensitivity should be evaluated after the control of blood glucose level in diabetic subjects. HOMA-IR can be used for evaluation of insulin resistance in patients on diet therapy or sulfonylureas [14] but might be not suitable for those on insulin therapy, because insulin treatment affects serum insulin levels, which in turn influences the feedback system between the liver and β cells. While it is necessary to evaluate insulin resistance in insulin users, HOMA-IR can only be used to evaluate insulin resistance in such patients after minimization of the effect of subcutaneously injected insulin.

In this study, insulin resistance was evaluated with HOMA-IR in patients on short acting insulin with or without sulfonylureas. The aim of this study was to validate HOMA-IR in patients with insulin-induced glycemic control. First, we treated patients with poor glycemic control with insulin. Then, we evaluated the agreement between HOMA-IR and clamp-IR of subjects on insulin therapy (Study 1). After confirming the validity of HOMA-IR in representing insulin resistance, we investigated the relationship between HOMA-IR and various clinical and biological parameters that are associated with diabetes to determine the clinical usefulness of HOMA-IR (Study 2).

Materials and Methods

Study 1

The study subjects were 19 Japanese type 2 diabetics [12 men and 7 women, aged 53.6±14.9 years, body mass index (BMI) 23.3±5.5 kg/m², hemoglobin A1c (HbA1c) 8.7±1.2 %] who had been admitted to Osaka University Hospital for glycemic control between 2001 and 2006. The clinical characteristics of the patients are summarized in Table 1. On admission, all oral hypoglycemic agents were withdrawn, and all subjects were treated with diet(25-30 kcal/kg standard body weight / day) and insulin (regular or ultrarapid insulin before each meal) for at least 2 weeks until fasting plasma glucose (FPG) fell to less than 140 mg/dL. NPH insulin was added before sleep in 10 subjects because their fasting plasma glucose was more than 140 mg/dL, though plasma glucose before sleep was less than 140 mg/dL. When FPG decreased to less than 140 mg/dL after treatment, insulin sensitivity was evaluated with HOMA-IR and clamp-IR. The correlation between HOMA-IR and M/I values derived from the standard euglycemic clamp was investigated.

HOMA-IR was calculated using the following formula: HOMA-IR = FPG (mg/dL) × fasting IRI (μU/mL)/405. Before HOMA-IR was calculated, patients were switched to treatment with sulfonylurea (glibenclamide 1.25 or 2.5 mg) instead of NPH insulin at the night of the day before the measurement to minimize the influence of insulin injected subcutaneously.

The euglycemic-hyperinsulinemic clamp was performed according to the method of DeFronzo et al. [3] with a little modification using an artificial pancreas (model STG-22, Nikkiso, Tokyo, Japan). Briefly, the test consisted of a 120-min euglycemic hyperinsulinemic clamp period. During the clamp test, subjects received primed-constant infusion of regular insulin (1.45 mU/kg min, Eli Lilly, Indianapolis, IN) and an exogenous glucose infusion to maintain blood glucose levels at 100 mg/dL and to achieve the desired steady-state serum insulin level (100 μU/mL). When the rate of exogenous glucose infusion reached a steady-state level, we evaluated insulin sensitivity as the average glucose infusion rate during the last 30 minutes divided by the average serum insulin level during the last 30 minutes (M/I).

Study 2

The study subjects were 156 Japanese with poorly controlled type 2 diabetes (79 men and 77 women) who had been admitted to Osaka University Hospital for glycemic control between 2001 and 2008. The clinical characteristics of the patients are listed in Table 2. Height and waist circumstance were measured in
HOMA-IR in insulin-treated diabetics

at 30 minutes after the 75g glucose load (Δinsulin 0-30 min / ΔPG 0-30 min).

Daily urine samples were collected for measurements of urinary CPR. Venous blood sample were collected before breakfast for measurements of LDL-cholesterol, HDL-cholesterol, triglyceride and adiponectin. Plasma adiponectin levels were determined with an adiponectin ELISA kit (Otsuka Pharmaceutical Co., Tokushima, Japan), as described previously [17]. The cases with insulin antibody that might have influence on glucose homeostasis were excluded from the studies.

Written informed consent was obtained from all subjects, and the study was approved by the ethics committee of Osaka University.

Table 2 Characteristics of the subjects of Study 2

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/females</td>
<td>156 (79 / 77)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.1±11.5</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.9±4.3</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>89.6±12.2 (n=136)</td>
</tr>
<tr>
<td>Estimated visceral fat area (cm²)</td>
<td>107.6±53.1 (n=102)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>127.7±17.5</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73.2±10.8</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>113.2±26.0</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>48.8±14.0</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>102.3±45.7</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.4±1.7</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL) after treatment</td>
<td>114± 18</td>
</tr>
<tr>
<td>Fasting immunoreactive insulin (μU/mL)</td>
<td>7.1± 5.1</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.0±1.3</td>
</tr>
<tr>
<td>Urinary C-peptide (μg/day)</td>
<td>65.4±44.6 (n=142)</td>
</tr>
<tr>
<td>ΔCPR (ng/mL)</td>
<td>2.2±1.2 (n=126)</td>
</tr>
<tr>
<td>Insulinogenic Index</td>
<td>0.20±0.25 (n=140)</td>
</tr>
<tr>
<td>adiponectin (μg/mL)</td>
<td>5.4±3.3</td>
</tr>
<tr>
<td>KITT (%/min)</td>
<td>1.92±1.22</td>
</tr>
</tbody>
</table>

Data are collected after glycemic control, except for HbA1c, and expressed means±SD.

HOMA-IR: homeostasis model assessment of insulin resistance, ΔCPR: increment of C-peptide from the glucagon stimulation test, KITT: K value from insulin tolerance test.
Results

Study 1

The mean insulin dose used to induce glycemic control was 27.2±27.9 U/day and FPG improved from 181.1±45.0 to 120.0±15.1 mg/dL. Ten subjects required NPH insulin for glycemic control, and sulfonylurea instead of NPH insulin was used at the night of the day before measurement of IRI and to calculate HOMA-IR. After treatment of patients with poor diabetic control with insulin, fasting IRI was 8.2±7.6 μU/mL and HOMA-IR was 2.45±2.38 (range: 0.77-9.01). M/I value derived from the standard euglycemic clamp test was 0.0464±0.0219 mg/kg/min/μU/mL (range: 0.0067-0.0976).

The correlation between log transformed HOMA-IR and log transformed M/I values derived from the standard euglycemic clamp was significant (r=-0.753, p=0.002, Fig. 1).

Study 2

After treatment, the mean fasting plasma glucose of 156 subjects improved from 178±51 to 114±18 mg/dL. The insulin dose used for glycemic control was 19.1±13.1 U/day. NPH insulin was used in 51 patients for glycemic control, sulfonylurea instead of NPH insulin was used at the night of the day before measurement of IRI and to calculate HOMA-IR. After treatment of patients with poor glycemic control, fasting IRI was 7.1±5.1 μU/mL and HOMA-IR was 2.0±1.3. In all of these patients, age (r=-0.292, p=0.0002), HDL-C (r=-0.342, p<0.0001), log transformed KITT (r=-0.264, p=0.0009), log transformed adiponectin (r=-0.309, p=0.0006) correlated negatively with log transformed HOMA-IR after glycemic control. On the other hand, BMI (r=0.499, p<0.0001), waist circumference (r=0.461, p<0.0001), eVFA (r=0.401, p<0.0001), diastolic blood pressure (r=0.223, p=0.0054), log transformed triglyceride (r=0.497, p<0.0001), urinary CPR (r=0.216, p=0.0099), ΔCPR (r=0.496, p<0.0001) and log transformed insulinogenic index (r=0.325, p=0.0002) correlated positively with the log transformed HOMA-IR (Fig. 2). Log transformed HOMA-IR did not correlate with systolic blood pressure, LDL-cholesterol or HbA1c (Table 3).

Discussion

FPG and serum insulin concentration are predominantly regulated by feedback loop between the liver and β cells [7]. Increased insulin resistance in the liver increases insulin secretion to stabilize hepatic glucose efflux. When the ability of β cells to secrete insulin is appropriate against insulin tolerance, plasma glucose level remains normal. However, defective β cell function results in increased hepatic glucose efflux and consequently leads to hyperglycemia. A rise in FPG from 80 to 140 mg/dL results in an increase in fasting plasma insulin, and increases in FPG beyond 140 mg/dL are
HOMA-IR in insulin-treated diabetics

Although the action of NPH insulin may last until the morning. To diminish the effect of exogenous insulin, NPH insulin was substituted with sulfonylurea at the night before the day of estimation of HOMA-IR. Treatment with sulfonylurea is considered to protect against damage of the feedback system between the liver and β cells. Indeed, Emoto et al. demonstrated that log transformed HOMA-IR correlated well with clamp associated with reduced insulin secretion and increased hepatic glucose output [18].

To evaluate insulin resistance with HOMA-IR, FPG should be less than 140 mg/d and the feedback system between the liver and β cells should be reconstructed. Injection of a high dose of insulin could affect fasting IRI and HOMA-IR. Regular or ultrarapid insulin injected before supper is almost cleared in the next morning, although the action of NPH insulin may last until the morning. To diminish the effect of exogenous insulin, NPH insulin was substituted with sulfonylurea at the night before the day of estimation of HOMA-IR. Treatment with sulfonylurea is considered to protect against damage of the feedback system between the liver and β cells. Indeed, Emoto et al. demonstrated that log transformed HOMA-IR correlated well with clamp...
IR in type 2 diabetics treated with sulfonylureas [14].

Insulin treatment may stimulate immunity, and antibodies to insulin may be produced in subjects treated with insulin. Therefore insulin users might have antibodies to insulin and these might have influence on glucose homeostasis. In this case, we cannot evaluate insulin sensitivity exactly. Before evaluating insulin sensitivity, we must consider whether insulin antibody is negative or not. The cases with insulin antibody that might have influence on glucose homeostasis should be excluded.

Study 1 showed significant correlation between log transformed HOMA-IR and log transformed M/I derived from the standard euglycemic clamp even in poorly controlled diabetic patients after treated with insulin. HOMA-IR correlated well with log transformed M/I in both highly insulin resistant subjects and low insulin resistant subjects. Furthermore, there was no difference in such relationship between patients who did not need and patients who needed NPH insulin for glycemic control. These results suggest that HOMA-IR appropriately expresses insulin sensitivity in type 2 diabetic patients under glycemic control with insulin when insulin regimen was optimized to evaluate the insulin sensitivity.

Insulin resistance correlates with obesity (especially visceral fat obesity)[19], hypertension [20], dyslipidemia [21] or hypoadiponectinemia [22, 23]. In Study 2, we have clarified the relationship between log transformed HOMA-IR or HOMA-IR and various clinical parameters. The same result was obtained when the subjects were restricted to insulin users. These parameters except HbA1c were evaluated after glycemic control, because it was presumed that the original state can be evaluated after correction of glucotoxicity.

Log transformed HOMA-IR correlated well with log transformed KITT. KITT is another method used to evaluate insulin sensitivity [16]. KITT is reported to be safe and reproducible method, and the values correlate well with M/I values derived from the euglycemic hyperinsulinemic clamp test [24, 25]. It should be emphasized that both KITT and HOMA-IR represent insulin sensitivity well even in poorly controlled diabetics after insulin treatment.

In this study, log transformed HOMA-IR correlated with various clinical parameters associated with obesity. BMI, waist circumstance and eVFA are parameters of body composition, HDL-C, diastolic blood pressure, TG and adiponectin are parameters associated with obesity. These results suggest that insulin resistance, expressed by HOMA-IR, is also associated with obesity in poorly controlled type 2 diabetic patients after insulin therapy. Although 51.1% of the patients were being treated with antihypertensive agents and 36.0% of the same subjects were being treated with hypolipidemic agents at study entry, HOMA-IR correlated with diastolic blood pressure, HDL-C and TG. These results emphasize the validity of HOMA-IR to reflect insulin resistance even after insulin treatment.

Log transformed HOMA-IR also correlated with various clinical parameters associated with insulin secretion. Urinary CPR, ΔCPR and insulinogenic index are parameters that express insulin secretion capacity. Increased insulin secretion seems to be also associated with obesity. Insulin can increase adiposity since it is a key hormone in adipogenesis. Age is also thought to correlate with insulin secretion capacity since insulin secretion ability is known to decrease with age [26]. This phenomenon is attributed in part to decreased β cell sensitivity to glucose-dependent insulinotropic polypeptide [27] and reduced β2-adrenergic receptor expression [28].

In non-diabetic subjects, increased insulin resistance increases insulin secretion to maintain plasma glucose level within the normal range. Increased insulin secretion might lead to increased adiposity, which enhances the likelihood of development of insulin resistance. In this regard, insulin secretion is reported to correlate with insulin sensitivity in a hyperbolic function in unrelated nondiabetic subjects [29]. However, when β cell fails to maintain insulin secretion against insulin resistance, relative insulin deficiency leads to impaired glucose tolerance or diabetes [1]. Diabetic subjects do not have adequate insulin secretion capacity to keep blood glucose within the normal range, but have insulin secretion capacity enough to enhance fat cell growth and body composition. This means that insulin secretion capacity relates to insulin resistance even in type 2 diabetic subjects. In this study, we showed that insulin resistance estimated by HOMA-IR correlated with insulin secretion ability estimated by urinary CPR, ΔCPR and insulinogenic index. This means that insulin secretion correlates with insulin sensitivity not only in nondiabetic subjects, but also in type 2 diabetic patients.

In diabetic patients with β cell dysfunction, HOMA-IR may not be accurate [10]. In the present study, insulin secretion ability expressed by ΔCPR of glucagon loading test was 2.1±1.0 ng/mL (range: 0.4-
HOMA-IR in insulin-treated diabetics

of HOMA-IR for the evaluation of insulin sensitivity in patients with poorly controlled type 2 diabetes after insulin therapy. The results also showed a close correlation between log HOMA-IR and log M/I values derived from the standard euglycemic clamp. Furthermore, HOMA-IR correlated with various clinical parameters even in patients with poorly controlled type 2 diabetes after glycemic control with insulin. These results suggest that HOMA-IR is a reliable and useful parameter for the evaluation of insulin sensitivity in patients with type 2 diabetes treated with insulin. Further examination is expected.

Appendix

We do not have any potential conflicts of interest relevant to this article.

References


4.8) in Study 1, and 2.2 ±1.2 (range: 0.4-5.6) in Study 2. FPG was controlled in all subjects within 140 mg/dL by insulin therapy with or without sulfonylureas. These findings suggest that we can evaluate insulin resistance with HOMA-IR in patients whose ΔCPR of glucagon loading test is more than 0.4 ng/mL and FPG was well controlled without long-acting insulin.

The insulin secretion capacity of Japanese subjects is lower than that of Caucasian subjects [30]. In Japanese subjects, the point of FPG beyond that insulin secretion reduces seems to be lower than that in Caucasian subjects. Reduced insulin secretion and increased hepatic glucose output may begin at the point of FPG lower than 140mg/dL. Further examination about the level of FPG on calculating HOMA-IR is expected.