Characterization of an Early Decline in Baseline Plasma Glucose Concentration after Acute Insulin Elevation during Euglycemic Hyperinsulinemic Clamp in Patients with Type 2 Diabetes Mellitus

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Abstract. To investigate the contribution of the liver to whole-body insulin resistance in patients with type 2 diabetes mellitus, we analyzed the early decline (slope "a") in the baseline plasma glucose level following acute hyperinsulinemia in the initial phase of a euglycemic hyperinsulinemic clamp study, rather than using an isotope-dilution method. Slope "a" was comparable among groups of diabetic and non-diabetic subjects, and did not correlate well with glucose infusion rate (GIR), an index of peripheral (primarily skeletal muscle) insulin resistance. In contrast, slope "a" was significantly lower in obese (BMI >25) type 2 diabetic patients compared with their non-obese counterparts, consistent with the general belief that obesity is a condition of insulin resistance in liver as well as in peripheral tissues. A subset of six insulin-resistant (nearly zero GIR) type 2 diabetic patients (pubertal adolescents) demonstrated a markedly blunted slope "a". Their insulin resistance (GIR) substantially recovered concomitant with an increase in slope "a" after pretreatment with somatostatin analogue in two cases studied, suggesting possible suppression of hepatic glucose production through lowering of plasma glucagon concentrations. Furthermore, slope "a" correlated significantly (r = -0.480, p <0.0001) with HOMA index (FPG × FIRI), the latter being recently regarded as an index of hepatic insulin resistance. These data showed that slope "a" obtained from euglycemic hyperinsulinemic clamp may be a clinically useful index of hepatic insulin resistance rather than an index of peripheral insulin resistance.

Key words: Hepatic insulin resistance, Type 2 diabetes mellitus, Euglycemic hyperinsulinemic clamp method

IT is well established that patients with type 2 diabetes mellitus are resistant to insulin-mediated glucose utilization [1-6]. While skeletal muscle is believed to be the major site responsible for insulin resistance [4], the contribution of the liver to whole-body insulin resistance is poorly understood. The liver handles glucose bidirectionally, that is, both by uptake and by release. Hepatic glucose uptake is less insulin sensitive than hepatic glucose production [4], thus the latter process is to a great extent implicated in insulin resistance. To date, however, hepatic insulin resistance in type 2 diabetes remains controversial; some investigators have found that the insulin-induced suppression of hepatic glucose output is impaired [5-9], whereas others have not confirmed these findings [3, 10]. In the euglycemic hyperinsulinemic clamp method, skeletal muscle glucose uptake can be assumed to approximate the exogenously infused glucose under a plasma concentration of insulin that allows the complete suppression of hepatic glucose output. Plasma insulin levels needed to suppress hepatic glucose production are substantially lower.
than those required to promote uptake by skeletal muscle: the half-maximally effective plasma insulin concentration (ED$_{50}$) lies around 70-110 μU/ml for stimulation of glucose disposal, but at only about 30 μU/ml (portal vein concentration) for suppression of hepatic glucose production [11], indicating that the liver is exquisitely sensitive to the inhibitory effects of insulin. Accordingly, our hypothesis is that acute elevation of plasma insulin concentrations in the basal state more profoundly affects the hepatic output of glucose than it does glucose disposal by skeletal muscle. If true, the rate of fall in the plasma glucose level during an initial phase of the euglycemic hyperinsulinemic clamp study can be a candidate variable for hepatic insulin resistance. In the present study, we tried to characterize this variable in relation to other clinical parameters.

Subjects and Methods

Subjects

Among subjects who had been examined using the euglycemic hyperinsulinemic clamp technique, results for 179 patients with type 2 diabetes were selected as representative of a well-performed clamp study. There were 123 men aged 13 to 71 (mean 47.8) years and 56 women aged 14 to 79 (mean 48.1) years. For comparison, data from 8 normal glucose-tolerant (NGT), 13 impaired glucose-tolerant (IGT), and 22 type 1 diabetic subjects were also included. Their demographic features appear in Table 1.

Methods

Euglycemic hyperinsulinemic clamp study

The method for the euglycemic hyperinsulinemic clamp study has been described [12]. In brief, after an overnight fast, a priming dose of short-acting insulin (Novolin R, Novo Nordisk A/S, Denmark) is infused at a stepwise decreased dose (from 3.56 mU/kg/min to 1.25 mU/kg/min) according to a format for 10 min, then followed by a constant infusion at the rate of 1.12 mU/kg/min. Such infusion quickly causes a peak in plasma insulin concentrations up to 150 μU/ml and then establishes a hyperinsulinemic plateau at 71.7 ± 31.9 μU/ml. The venous plasma glucose level is maintained at 80 mg/dl by varying infusions of 10% glucose, according to the algorithm described by DeFronzo et al. [13]. The average glucose infusion rate (GIR), a measure of in vivo resistance (or sensitivity) to insulin-stimulated glucose utilization, was determined during the final 30 min of a 60 to 90 min steady-state euglycemia.

Determination of initial rate of decline (slope “a”) in basal plasma glucose level

After the primed-continuous insulin infusion, the basal plasma glucose level declined linearly to the

| Table 1. Demographic features of the 222 subjects |
|-----------------|------|-------|-----------------|-----|
|                | NGT  | IGT   | Type 1 DM       | Type 2 DM |
| n (M : F)      | 8 (5 : 3) | 13 (8 : 5) | 22 (6 : 16)    | 179 (123 : 56) |
| Age            | 33 ± 7 | 48 ±15a | 36 ±14b       | 48 ± 17h,c |
| BMI (kg/m²)    | 21.2 ± 2.3 | 22.9 ± 3.4 | 21.7 ± 3.2 | 23.4 ± 4.1 |
| FPG (mg/dl)    | 95 ± 2 | 124 ±24a | 173 ±44b    | 158 ± 47a |
| HbA₁c (%)      | 4.7 ± 0.3 | 5.5 ± 0.5a | 9.6 ± 2.7n,b  | 9.0 ± 2.9n,b |
| FIRI (μU/ml)   | 8.0 ± 3.5 | 8.1 ± 4.2 | —             | 10.5 ± 6.5n,b |
| TG (mg/dl)     | 94 ± 88 | 164 ± 80 | 95 ± 54b     | 190 ± 16c |
| FFA (mEq/L)    | 0.52± 0.20 | 0.60± 0.39 | 0.73± 0.62a  | 0.69 ± 0.43c |
| GIR (mg/kg·min)| 6.1 ± 1.6 | 5.5 ± 2.4 | 4.5 ± 2.4    | 3.5 ± 2.0p,b,c |

NGT = normal glucose tolerance, IGT = impaired glucose tolerance
a, b, c = significance (p < 0.05) vs. NGT, IGT, Type 1, respectively
BMI = body mass index, FPG = fasting plasma glucose, HbA₁c = glycated hemoglobin A
FIRI = fasting immunoreactive insulin, TG = triglycerides, FFA = free fatty acids, GIR = glucose infusion rate
clamp level of 80 mg/dl. The change in plasma glucose concentration was measured automatically every 5 min by use of the artificial endocrine pancreas (Nikkiso STG-22, Nikkiso Co., Tokyo). Five consecutive values for plasma glucose concentration during the initial phase of decline were used to construct a linear curve by means of least-squares regression analysis. The linearity of the slope was estimated from the coefficient of correlation (r) for consecutively determined plasma glucose levels. In this study, all subjects had r values greater than 0.95. The slope of this line was designated as “a” and was expressed as a positive value.

Other determinations

Plasma insulin levels were determined using a commercial kit (Phadeseph Insulin RIA, Pharmacia Diagnostics, Sweden).

Statistical analysis

Data were presented as means±SDM. Statistical analysis was performed using Scheffe’s one-way ANOVA. The evaluation of correlation utilized Pearson’s coefficient of correlation.

Results

1) Slope “a” in groups of subjects with varying degrees of glucose tolerance

The mean values for slope “a” (mg/dl/min) were comparable, with no significant differences found between any two groups: 1.375±0.441 for IGT (n=13), 1.377±0.362 for type 1 diabetes (n=22) and 1.310±0.556 for type 2 diabetes (n=179) and a moderately high 1.668±0.282 for NGT (n=8). When type 2 diabetic patients were categorized into three groups according to body mass index (BMI), the mean slope “a” was significantly smaller in the obese group (BMI>25) than in the two non-obese groups (1.137±0.353 vs. 1.539±0.664 and 1.378±0.593, p<0.01) (Fig. 1). There were no significant differences in fasting plasma glucose concentrations among these groups.

2) Correlation between slope “a” and GIR

When the type 2 diabetic patients were divided into two groups according to GIR value, there was a weak correlation (r=0.387, p<0.005) between slope “a” and GIR in the subgroup of 67 insulin-sensitive (GIR>4.0 mg/kg/min) patients (Fig. 2-A). Such a correlation was not found (r=0.206, p>0.05) in the subgroup of 112 insulin-insensitive (GIR<4.0) type 2 diabetic patients (Fig. 2-B).

3) Slope “a” in a subset of young type 2 diabetic patients with severe insulin resistance

Table 2 shows the results from six young type 2 diabetics who were demonstrated to have severe insulin resistance by the euglycemic hyperinsulinemic clamp study. Figs. 3 and 4 depict the representative results of the clamp study performed in patients 1 and 2, showing extremely low GIR (0.19 and 0 mg/kg/min) and a markedly blunted slope “a” (1.016 and 0.844 mg/dl/min), respectively. In these cases, despite continuous insulin infusion (1.12 mU/kg/min) the plasma glucose level remained almost constant at around the clamp level without supplying any exogenous glucose, suggesting the persistent
Fig. 2. Correlation between slope “a” and glucose infusion rate (GIR) in the insulin-sensitive subgroup (GIR > 4.0 mg/kg/min, n=67) (A) and in the insulin-insensitive subgroup (GIR < 4.0 mg/kg/min, n=112) (B) of type 2 diabetic patients. Dotted lines represent 95% confidence intervals.

Table 2. Six cases of severe insulin-resistant type 2 diabetes

<table>
<thead>
<tr>
<th>Case</th>
<th>BMI (kg/m²)</th>
<th>HbA1c (%)</th>
<th>FPG (mg/dl)</th>
<th>FIRI (CPR)</th>
<th>GIR (mg/kg/min)</th>
<th>Slope “a”</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) M.Y. (16, F)</td>
<td>25.2</td>
<td>10.4</td>
<td>227</td>
<td>—</td>
<td>0.19</td>
<td>1.016</td>
<td>Ins</td>
</tr>
<tr>
<td>2) N.S. (18, F)</td>
<td>19.2</td>
<td>12.5</td>
<td>214</td>
<td>(1.3)</td>
<td>0</td>
<td>0.844</td>
<td>Ins</td>
</tr>
<tr>
<td>3) K.S. (13, M)</td>
<td>19.1</td>
<td>10.9</td>
<td>180</td>
<td>17.2</td>
<td>0.34</td>
<td>1.066</td>
<td>OHA</td>
</tr>
<tr>
<td>4) N.T. (16, M)</td>
<td>30.4</td>
<td>9.1</td>
<td>124</td>
<td>—</td>
<td>0</td>
<td>1.066</td>
<td>Diet</td>
</tr>
<tr>
<td>5) T.H. (14, M)</td>
<td>24.3</td>
<td>11.7</td>
<td>237</td>
<td>10.1 (1.7)</td>
<td>0.82</td>
<td>1.294</td>
<td>Ins</td>
</tr>
<tr>
<td>6) M.Y. (17, F)</td>
<td>25.5</td>
<td>14.9</td>
<td>217</td>
<td>6.0</td>
<td>0.87</td>
<td>1.092</td>
<td>Ins</td>
</tr>
</tbody>
</table>

GIR: mg/kg/min, Slope “a”: mg/dl/min, FPG, fasting plasma glucose (mg/dl), OHA, oral hypoglycemic agents, Ins, insulin, FIRI, fasting immunoreactive insulin (pU/ml), CPR, fasting C-peptide immunoreactivity (ng/ml)

Fig. 3. Euglycemic hyperinsulinemic clamp study in a patient with MODY (16 year old girl, BMI 25.2). This case (♀1) took about 3 hours following the initiation of insulin infusion to reach the clamp level and thereafter only a negligible amount of glucose was needed to maintain euglycemia over the next 3 hours. slope “a”=1.016 mg/dl/min, GIR=0.19 mg/kg/min
unrestrained release of glucose from the liver. On a separate day, these patients received a subcutaneous injection of somatostatin analogue octreotide 50 μg, 2 hours prior to the clamp study, and then an identical clamp study was performed (data not shown). In the 2nd study, slope “a” in each case increased to 1.667 and 1.529, and GIR to 2.67 and 1.80, respectively.

4) Correlation between slope “a” and HOMA index

We correlated slope “a” with HOMA index (FPG x FIRI), which has been published as being a good proxy of insulin action [14], in 106 non-insulin treated subjects with type 2 diabetes and IGT in whom fasting plasma insulin values were available. As shown in Fig. 5, a significant correlation was found (r = -0.480, p < 0.0001).

Discussion

It has been indicated that some type 2 diabetic individuals have a predominant defect in hepatic insulin sensitivity and less or near-normal peripheral (primarily muscle) insulin sensitivity, and vice versa [15]. Therefore, separate determinations of insulin resistance with no employing isotope tracer (if possible) could contribute to a profound understanding of pathophysiology of type 2 diabetes. In this context, we encountered a unique subset of severe insulin-resistant type 2 diabetic adolescents, who showed very small values of slope “a”, together with nearly

![Fig. 4. Euglycemic hyperinsulinemic clamp study in a type 2 diabetic patient (18 year old girl, BMI 19.2). This case (#2) took about 3 hours following the initiation of insulin infusion to reach the clamp level and thereafter no exogenous glucose was needed to maintain plasma glucose at 98–100 mg/dl for 60 min and for another 30 min on doubling the insulin infusion rate. slope “a” = 0.844 mg/dl/min, GIR = 0 mg/kg/min.](image)

![Fig. 5. Correlation between slope “a” and HOMA index [(fasting plasma glucose in mg/dl) x (fasting plasma insulin in μU/ml)] in 106 subjects with type 2 diabetes and IGT.](image)
zero GIRs. In these cases, if hepatic glucose output is completely blocked, plasma glucose level can't maintain euglycemia without a supply of exogenous glucose over 2 to 3 hours as seen in Figs. 3 and 4, because insulin-independent tissues, such as brain, liver (in terms of glucose uptake), kidney and erythrocytes, utilize roughly 70% of total basal glucose disposal [16]. In severe insulin resistant state, insulin-dependent tissues (skeletal muscle, adipose tissue and liver) use only limited glucose despite the presence of hyperinsulinemia, while brain and other insulin-independent tissues constantly consume a certain amount of glucose (approximately 6 g/h) which is largely supplied by the liver [17]. Thus, these findings strongly suggested some amount of unrestrained glucose release from the liver and tempted us to interpret this unusual adolescent group as having severe insulin resistance of the liver. Furthermore, treatment with somatostatin analogue prior to the clamp study resulted in demonstrable improvement of both slope "a" and GIR, probably due to suppression of hepatic glucose production through inhibiting plasma glucagon levels. Somatostatin so far has not been known to affect peripheral insulin sensitivity other than suppressing counterregulatory hormones such as glucagon and growth hormone, thus it is currently used in SSPG method, another measure of insulin resistance [18]. Therefore, the substantial recovery of GIR seems to be secondary to the effect of somatostatin on the liver, because more intensive suppression of hepatic glucose production per se apparently raises GIR value. Therefore, these observations appear to favor the view that the slope "a" represents largely the degree of insulin resistance of the liver rather than the skeletal muscle. In the present study, we examined whether the slope "a" could be a measure reflecting insulin resistance of the liver. In our clamp study, insulin priming was achieved in an identical manner, so we can compare the slope "a" between individuals. The present findings demonstrated that there was neither a difference in the mean value of slope "a" between groups of diabetic and non-diabetic subjects, nor significant correlation of slope "a" to GIR in the majority of type 2 diabetic patients. These results were unexpected because we simply believed that the rate of the decline in baseline plasma glucose concentrations after acute hyperinsulinemia depends on the rate of peripheral glucose utilization (GIR). The baseline plasma glucose level is determined by the balance between hepatic glucose production and glucose disposal largely by insulin-independent tissues. In such basal state, an acute elevation of plasma insulin concentration could have a greater effect on the liver than on the skeletal muscle, not only because the former's ED₅₀ of insulin is lower than that for latter, but also because the relationship between activation and deactivation times of insulin is different for the liver (in terms of suppression of glucose release) and for peripheral tissue (as stimulation of glucose uptake), that is, at any insulin level, the liver is activated more rapidly and persistently than skeletal muscle [19]. The other finding that slope "a" was small in the obese group compared with their non-obese counterparts is in line with previous reports showing that obese subjects are insulin resistant both in the liver and in periphery [4, 8, 20]. Finally, the behavior of slope "a" in terms of correlation to GIR was similar to that of the HOMA index. That is, in the subjects with higher GIR or NGT, both slope "a" and the HOMA index showed a significant correlation to GIR, while in subjects with lower GIR or type 2 diabetes, the correlation was poor [21]. This may imply that both indices (slope "a" and HOMA index) have a similar physiological meaning. This assumption was further supported by the present findings that both were significantly correlated with one another. HOMA index consists of (FPG) × (FIRI), in which fasting plasma glucose is well known to correlate with hepatic glucose output [3, 5, 22], and fasting plasma insulin levels potentially relate to the hepatic clearance of insulin. From these considerations, we believe that HOMA index represents hepatic insulin resistance rather than a skeletal muscle insulin resistance. Recently, DeFronzo's group made a similar assumption based on their results that the product of basal endogenous glucose production measured with 3-[³H] glucose and fasting plasma insulin concentration (a direct measure of hepatic resistance to insulin) correlated well (r = 0.69, p < 0.0001) with HOMA [15]. Therefore, taken together, it seems plausible to consider that slope "a" in the euglycemic hyperinsulinemic clamp study is a candidate variable reflecting the degree of hepatic resistance to insulin, even though a part of it can be attributed to the peripheral effect of insulin. The minimum model method also analyzes the slope of decline of plasma glucose level after in-
travenous glucose, but not the slope of decline in baseline plasma glucose level after insulin infusion. Hyperglycemia per se can effectively inhibit hepatic glucose output independently of insulin, thus both measures represent different physiological meanings.

The major limitation of the present work, however, is that there is no experimental basis supporting slope “a” to be a measure of hepatic insulin resistance. Certainly further studies are needed to prove this clinically interesting issue.

References