OBSERVATIONS

Plasma Adrenomedullin Levels in Patients With Diabetes

The novel hypotensive peptide, adrenomedullin (AM), was originally discovered in human pheochromocytoma (1). The major source of circulating AM is considered to be the vasculature (2), and this peptide may act as an antiproliferative factor for vascular smooth muscle cells (3). Diabetes is characterized by long-term complications, including retinopathy, nephropathy, and neuropathy, all of which are closely related to vascular damage; the role of AM in diabetes has been investigated in this context. Insulin secretion in the pancreas is inhibited by AM, an intravenous injection of which reduces the plasma insulin concentration (4). Hyperglycemia increases vascular AM expression through a protein kinase C (PKC)-dependent pathway (5). The issue of whether plasma levels of AM in diabetic patients with normal renal function are increased remains controversial (6–8). When evaluating the relationship between plasma levels of AM and glucose, patients with renal impairment should be excluded, because plasma AM levels in those patients with mild renal insufficiency and serum creatinine levels of 1.34 ± 0.10 mg/dl are three times higher than those in control individuals (9). We examined whether or not plasma AM levels are related to clinical parameters, including levels of plasma glucose and HbA1c, in diabetic patients without nephropathy through the use of a novel immunoradiometric assay (10).

A total of 77 diabetic patients (mean age 60.4 ± 1.3 years, 30 men and 47 women) and 15 healthy individuals (mean age 35.2 ± 3.7 years, 7 men and 8 women) participated in the study. Patients with serum creatinine levels >1.2 mg/dl, urinary protein that was positive according to the dip-stick test, or urinary albumin that was ≥30 mg/day were excluded. Blood samples were taken from the antecubital vein into chilled polypropylene tubes containing EDTA-2Na (1 mg/ml of blood) and aprotinin (500 U/ml of blood) early in the morning after an overnight fast. Plasma was obtained by immediate centrifugation at 4°C and 3,000 rpm for 15 min. Plasma concentrations of AM were measured using the AM RIA Shionogi kit (Osaka, Japan). Levels of plasma glucose and HbA1c in the patients were significantly higher than those in control subjects (132.1 ± 5.3 vs. 86.9 ± 2.2 mg/dl, P < 0.001 and 6.40 ± 0.17 vs. 4.92 ± 0.05%, P < 0.001, respectively). There were no significant differences in plasma AM levels, serum creatinine levels, mean blood pressure, and BMI between the patients and control subjects (11.0 ± 0.3 vs. 10.6 ± 0.5 fmol/ml, 0.73 ± 0.02 vs. 0.70 ± 0.04 mg/dl, 90.8 ± 1.3 vs. 92.3 ± 2.9 mmHg, and 22.9 ± 0.3 vs. 23.0 ± 0.8 kg/m2, respectively). The AM plasma concentration in all participants was 10.9 ± 0.2 fmol/ml, which significantly correlated with the values for mean blood pressure (r = 0.32, P < 0.01) and BMI (r = 0.26, P < 0.05) but not with those for age and levels of serum creatinine, plasma glucose, and HbA1c.

Hayashi et al. (6) reported that plasma AM levels in patients with poorly controlled diabetes are significantly higher than those in healthy volunteers and proposed that the elevated plasma AM level originates from vascular AM expression induced by hyperglycemia (5). However, two other groups reported that plasma AM levels are not affected by plasma glucose levels (7,8). The influence of nephropathy must be excluded when evaluating the relationship between plasma levels of AM and glucose, because the plasma AM level is closely associated with renal function (9). The results of the present study showed that plasma AM levels and blood glucose controls do not significantly correlate in strictly selected patients without nephropathy. Thus, it is likely that plasma AM levels are not directly affected by plasma glucose levels. However, vascular AM induced by hyperglycemia (5) may act as a local factor unless plasma levels of AM are increased. Ishimitsu et al. (11) suggested that the increased plasma AM level associated with essential hypertension and chronic renal failure is involved in the defense mechanism against further blood pressure elevation and/or body fluid retention (11). The significant correlation between plasma AM levels and mean blood pressure found in this study indicates that AM participates in this defense mechanism in diabetic patients.

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References

Differential Antioxidant Status Among Indo-Asians Compared With Caucasians With and Without Diabetes

Antioxidant status is important in the pathogenesis of endothelial damage and diabetes-related vascular complications (1), which are more prevalent in the U.K. Indo-Asian population compared with Caucasians (2). We have studied Indo-Asian and Caucasian patients with type 2 diabetes and race-matched nondiabetic control groups, excluding those subjects with any other significant medical conditions or with advanced diabetes-related complications.

Assays in serum from nonfasting blood were undertaken for vitamin A as retinol, vitamin E as α-tocopherol standardized against the total cholesterol level, vitamin C as ascorbate as previously described (3), and total antioxidant activity (TAA) as the ferric reducing ability of plasma (FRAP) (4). The HbA1c normal local range is 3.8–5.5%. Statistical analysis was achieved through one-way analysis of variance followed by the unpaired Student’s t test, the χ2 test, and multiple regression analysis.

There were no significant differences between the groups in age, sex, and BMI. Diabetes duration (13.4 ± 11.1 vs. 8.3 ± 7.3 years, P < 0.05) and smoking prevalence (16 vs. 4%, P < 0.001) were significantly higher among the Caucasian patients. Vegetarianism was significantly more prevalent among the Indo-Asians patients (30 vs. 2%, P < 0.001).

Significant differences between the groups were found in levels of HbA1c, serum vitamin C, and serum vitamin A, but not serum vitamin E or TAA (Table 1). In multivariate analysis, TAA was related to sex, but not age, smoking status, ethnicity, or HbA1c levels (P < 0.05). When comparing Caucasian with Indo-Asian nondiabetic subjects, vitamin C levels were significantly lower in Indo-Asian patients, but their vitamin A levels were significantly higher. Caucasian patients had significantly lower vitamin A levels compared with Caucasian nondiabetic subjects. Among the Indo-Asian patients, both vitamin C and vitamin A levels were significantly lower than in the control Indo-Asian patients. No significant differences were found between the Caucasian and Indo-Asian patients. In multivariate analysis, which included analysis of age, sex, ethnicity, smoking status, vegetarian diet, and HbA1c levels as independent variables, vitamin C correlated with ethnicity and HbA1c levels (P < 0.01), and HbA1c concentration alone was a significant variable for vitamin A (P < 0.01). However, when the presence of diabetes is considered as a variable, both vitamins correlate to this (P < 0.001), and the significant effect of HbA1c concentration as an independent variable is lost.

Indo-Asian patients, as compared with race-matched nondiabetic subjects, showed a significant reduction in levels of both vitamin C and vitamin A, and potential confounding factors did not relate to the observed differences. Compared with their race-matched control group, Indo-Asian patients appeared to lose the potential protective effect of both vitamins, which may possibly contribute to enhanced vascular risk.

Low levels in the blood of both vitamin A and vitamin C are associated with an increased risk of cardiovascular disease (5,6), although vitamin A is not classified as an antioxidant. Recent studies have shown that the reduced circulatory status of vitamin A in diabetic animals may be secondary to an impaired transport mechanism (7) and that the plasma concentrations of the carrier proteins of vitamin A, retinol-binding protein, and transthyretin, may be decreased in human diabetes (8). Similarly, low vitamin C levels may be associated with the diabetic state itself (9), because an impairment in ascorbic acid metabolism and intracellular transport that is not correctable with dietary supplementation of vitamin C may exist (10).

The results suggest an association among ethnicity, diabetes, and antioxidant status that requires further evaluation as a factor that determines potential risk of differential diabetes-related vascular complications.

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References

Table 1—Levels of HbA1c, serum individual antioxidants, and total antioxidant activity among Caucasian and Indo-Asian patients and race-matched nondiabetic control subjects

<table>
<thead>
<tr>
<th></th>
<th>Caucasian study population</th>
<th>Indo-Asian study population</th>
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<tbody>
<tr>
<td></td>
<td>Control patients</td>
<td>Diabetic patients</td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>50</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.5 ± 0.4</td>
<td>7.4 ± 1.5*</td>
</tr>
<tr>
<td>Vitamin C (µmol/l)</td>
<td>89 ± 26</td>
<td>60 ± 22*</td>
</tr>
<tr>
<td>Vitamin A (µmol/l)</td>
<td>2.0 ± 0.9</td>
<td>1.8 ± 0.8</td>
</tr>
<tr>
<td>Vitamin E (µmol/l)</td>
<td>22.7 ± 10.2</td>
<td>24.2 ± 9.7</td>
</tr>
<tr>
<td>FRAP (µmol [Trolox equivalent])</td>
<td>551 ± 215</td>
<td>656 ± 327</td>
</tr>
</tbody>
</table>

Data are means ± SEM. *P < 0.001 vs. Caucasian control subjects; †P < 0.001 vs. Indo-Asian control subjects; §P < 0.05 vs. Indo-Asian control subjects.
The Batista Procedure Significantly Improved Cardiac Function and Glycemic Control in a Diabetic Patient With Severe Insulin Resistance

E novo insulin resistance (IR) is a major metabolic defect in type 2 diabetes (1–3). In addition to this inherent defect, other temporary endogenous and exogenous factors result in various degrees of IR. Endogenous factors, such as catecholamines and glucocorticoids, are collectively referred to as counterregulatory hormones; exogenous factors include iatrogenic diabetogenic agents, such as steroids. Catecholamines, and particularly norepinephrine, are produced in excessive amounts, both locally and systemically, during heart failure (4,5). In diabetic patients with advanced heart failure, glycemic control is difficult to attain, and, consequently, requirements for insulin or oral hypoglycemic agents are increased. Heart failure secondary to advanced dilated cardiomyopathy of various etiologies may become refractory to medical therapy, and effective surgical intervention was, until recently, limited to cardiac transplantation (6,7). The Batista procedure, which is a relatively new operation that involves a partial left ventriculectomy, was pioneered by Dr. Randas Batista in Brazil in the mid-1990s (8). Recent related literature provides the proven efficacy of this novel procedure to alleviate the crippling symptoms of advanced dilated cardiomyopathy (9–11).

It is logical to speculate that improving cardiac function and, thus, reducing circulating catecholamines would improve “temporary” IR in diabetic patients with severe cardiomyopathy. When medical therapy fails and the patient’s condition requires cardiac transplantation, the Batista procedure provides a valuable treatment option to improve cardiac function. An additional advantage of the procedure is improved glycemic control and increased insulin sensitivity.

We report here the case of a 54-year-old obese woman with long-standing insulin-requiring type 2 diabetes with insulin resistance. She had coronary artery disease manifested by frequent angina and experienced episodes of acute heart failure on a weekly basis. In addition, she had class III cardiac symptoms, as defined by the New York Heart Association. Cardiac work-up revealed dilated ischemic three-vessel disease cardiomyopathy with ejection fraction (EF) of <15%. She was maximized on antifailure and anti-ischemic medical therapy, including furosemide, long-acting and sublingual nitrates, digoxin, and ACE inhibitors. Her sugar levels were controlled with administration of >100 U insulin daily; this dose was achieved with the use of troglitazone, administration of which lowered the initial insulin requirements. She was considered a nonsurgical candidate for coronary bypass because of advanced cardiomyopathy. She was then referred to a cardiovascular surgeon who trained under Dr. Batista, and he performed the combined Batista procedure and coronary bypass operation. The patients cardiac function improved dramatically after the operation. Symptoms related to heart failure and ischemia resolved, and EF improved to 35%. All of her medications were reduced or discontinued. Additionally, her insulin requirements decreased by >50%.

We believe that the patient achieved reduced levels of local and circulating catecholamines, resulting from improved cardiac function that was brought about by surgery. This presumed reduction in circulating catecholamines, we believe, improved glycemic control by reversing temporary IR, a condition that results from the counterregulatory effects of these catecholamines.

References

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Letters
Elevated Serum Content of Macrophage Migration Inhibitory Factor in Patients With Type 2 Diabetes

Macrophage migration inhibitory factor (MIF) is rediscovered as a proinflammatory cytokine and glucocorticoid-induced immunoregulator (1). We found that MIF was expressed in adipocytes, and that excretion of MIF protein could be induced by tumor necrosis factor-α (TNF-α) stimulation (2). Interestingly, it has been reported that the insulin secretion is regulated by the glucose-dependent production of islet β-cell MIF (3). According to this report, MIF mRNA expression in the islet β-cells is increased when they were incubated in higher glucose concentrations, and, moreover, islet MIF stimulates insulin secretion in a concentration-dependent manner. In this study, we examined the concentration of serum MIF in type 2 diabetes to clarify the possibility that MIF is associated with the disregulation of glucose metabolism.

In the current study, 79 patients with type 2 diabetes and 79 age- and sex-matched normal healthy control subjects participated. The characteristics of the subjects are shown in Table 1. Informed consent was obtained from all subjects participating in this investigation. Of the 79 diabetic subjects, 30 were taking oral hypoglycemic agents, 32 were injecting human insulin, and 17 had diet therapy only. Specialized ophthalmologists diagnosed the diabetic retinopathy and its clinical stage. The concentrations of serum MIF were measured according to a method previously described (2).

The content of serum MIF in type 2 diabetic patients was significantly high compared with that in normal control subjects, showing 20.7 ± 13.3 and 5.2 ± 3.0 ng/ml for type 2 diabetes and control subjects, respectively (P < 0.0001) (Fig. 1).

Table 1—Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Type 2 diabetes</th>
<th>Control subjects</th>
<th>P</th>
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<tbody>
<tr>
<td>n</td>
<td>79</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.8 ± 9.6</td>
<td>53.8 ± 9.6</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>53/26</td>
<td>53/26</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 ± 2.9</td>
<td>23.8 ± 3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>101 ± 13</td>
<td>103 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td>White blood cell count (/ml)</td>
<td>6,462 ± 1,809</td>
<td>6,077 ± 1,754</td>
<td>NS</td>
</tr>
<tr>
<td>Red blood cell count (×10¹²/ml)</td>
<td>453 ± 65</td>
<td>451 ± 39</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet (×10⁹/ml)</td>
<td>21.7 ± 6.7</td>
<td>22.9 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>195 ± 36</td>
<td>207 ± 29</td>
<td>NS</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>120 ± 65</td>
<td>126 ± 111</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>53 ± 20</td>
<td>56 ± 16</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>145 ± 41</td>
<td>100 ± 13</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are n or means ± SD.

Figure 1—Serum content of MIF in patients with type 2 diabetes. *Statistical significance compared with that in normal subjects (P < 0.0001).
tissue through the action of MIF itself and/or the induction of TNF-α. Abundant quantities of MIF were demonstrated in the endocrine pancreas by immunohistochemical analysis, suggesting a role for MIF in glucose metabolism (4). Differentiated insulin-secreting β-cell line INS-1 had the potential to express MIF, and the MIF expression was potentiated by the glucose concentration in culture media. Moreover, in perfusion studies performed with isolated rat islets, immunoneutralization of MIF reduced the first and second phases of the glucose-induced insulin secretion response by 39 and 31%, respectively. It is speculated that MIF stimulates insulin secretion and MIF secretion is regulated by glucose. It may be reasonable that MIF seems to modulate the carbohydrate metabolism as MIF modulates the inflammatory and immunological responses, counterregulating impaired homeostasis by the action of glucocorticoid suppression (5).

It should be noted that serum MIF content showed correlation with diabetic retinopathy. TNF-α and interleukin-1 (IL-1) play an important role in early diabetic retinopathy (6,7). In proliferative retinopathy, vascular epidermal growth factor and transforming growth factor-β are more essential. MIF induces the production of TNF-α and IL-1 and they also induce the MIF production in the retina. We consider an elevated level of MIF in the early stage of diabetic retinopathy to reflect the enhancement of IL-1 and/or TNF-α.

Increased serum MIF may be another nonspecific marker for illness in general, rather than a key player in the pathogenesis of type 2 diabetes. In fact, MIF was increased in the sera of patients with uveitis and atop dermatitis (8,9). However, MIF has two biological effects: insulin secretion and TNF-α induction, and insulin resistance is closely related to inflammation. Further examination will be needed to know whether elevated serum MIF is associated with the pathogenesis of type 2 diabetes.

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References

Table 2—Correlations between serum MIF and diabetic characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>r</th>
<th>P</th>
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<tr>
<td>Fasting plasma glucose</td>
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<td>NS</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes duration</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Dose of insulin</td>
<td>0.08</td>
<td>NS</td>
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</tbody>
</table>

Figure 2—The changes of serum MIF content with diabetic complication. A: retinopathy; B: nephropathy; C: neuropathy. *Statistical significance compared with that in patients without retinopathy (P < 0.05).
Lamin A/C Mutation in a Woman and Her Two Daughters With Dunnigan-Type Partial Lipodystrophy and Insulin Resistance

The lipodystrophies are a heterogeneous group of disorders that are characterized by the complete or partial absence of adipose tissue, and they occur with a variety of clinical manifestations that include marked insulin resistance and diabetes (1). Patients with autosomal dominant Dunnigan-type familial partial lipodystrophy (FPLD) (OMIM 151660) are born with normal adipose tissue distribution, but, after puberty, they experience adipocyte degeneration in their extremities, trunk, and gluteal region (1-4). Subjects with FPLD have insulin resistance before the development of diabetes, which is often associated with dyslipidemia and early coronary heart disease (CHD). Recently, the FPLD gene was mapped to chromosome 1 q21-22 (1-4). This chromosomal segment harbors the LMNA gene, which encodes the nuclear structural proteins lamin A and C (1-4). Mutations in LMNA were shown to underlie autosomal dominant—Emery-Dreifuss muscular dystrophy (AD-EDMD), which is characterized by regional and progressive myocyte degeneration (5). We surmised that the analogy between the regional myocyte degeneration in AD-EDMD and the regional adipocyte degeneration in FPLD and its chromosomal position made LMNA a good candidate gene for FPLD.

The proband was a 56-year-old woman of northern European descent. She developed diabetes at the age of 30 years and was initially treated with oral hypoglycemic agents. At age 35, she presented with mild hypertriglyceridemia and had biopsy-confirmed fatty infiltration of the liver. At that time, the clinical diagnosis of partial lipodystrophy was made based on the characteristic clinical features, which included the absence of subcutaneous fat tissue from her upper and lower extremities, her extremely muscular appearance, which commenced in adolescence, and the presence of excess adipose tissue in her face and neck, which gave her a pseudo-Cushingoid appearance.

Her glycemic control became progressively more difficult, and, at age 45, insulin therapy was initiated. At age 52, she underwent triple-vessel bypass coronary arterial grafting for unstable angina. She is presently treated with 30 U daily of human insulin with standard medications, including atorvastatin, for secondary prevention of CHD. Her 37- and 35-year-old daughters were each diagnosed with FPLD in their early twenties, based on their characteristic physical features, which were identical to those of their mother. Both daughters developed elevated plasma triglycerides in their early thirties. Both daughters had marked elevations of serum insulin and C-peptide, both of which exceeded twice the upper limit of normal, although neither daughter is yet to be diagnosed as hyperglycemic.

Primers for DNA amplification and sequencing were derived using published sequence information for all 12 exons, all intron-exon boundaries, and the 5’- and 3’-untranslated regions of LMNA (6). DNA sequencing revealed that the proband was heterozygous for a G → A change at codon 482 in exon 8, which predicted the replacement of arginine (CGG) by glutamine (CAG). A normal control subject was homozygous for the wild-type sequence. We found no other LMNA coding or flanking sequence abnormalities. A rapid genotyping assay was then developed, which revealed that both daughters were heterozygous for the LMNA R482Q mutation, whereas the mutant allele was absent from 276 normal Caucasians (P < 2.8 × 10⁻⁷).

These results strongly suggest that the LMNA R482Q mutation is the molecular basis for FPLD in these women. Lamin A and C are members of the intermediate filament multigene family. Both are absent from early embryos and undifferentiated cells but are present in most terminally differentiated cells, including adipocytes. Lamin A and C form dimers through their rod domains and interact with chromatin and integral proteins of the inner nuclear membrane through binding sites located in both the rod domain and in the 3’-terminal globular tail (6). The nonconservative LMNA R482Q change occurs within the 3’-terminal tail sequence that is common to both lamin A and C (6). The evolutionary conservation of R482 across human, mouse, rat, and chicken suggests that this residue is important for the normal function of lamin A and C (6). Given the strong genetic association of LMNA R482Q with FPLD, it now becomes important to demonstrate that other FPLD subjects also have LMNA mutations.

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Novel concepts and materials derived from this work have been embodied in U.S. patent application C99-482 (17 September 1999).
Effect of Bezafibrate on Insulin Sensitivity in Nonobese Japanese Type 2 Diabetic Patients

There are two subtypes in Japanese nonobese type 2 diabetic patients: one with normal peripheral insulin sensitivity and the other with primary peripheral insulin resistance (1,2). We recently showed that insulin resistance observed in nonobese Japanese type 2 diabetic patients is positively correlated with fasting triglyceride levels but not with BMI (3). There is a possibility that disturbed triglyceride metabolism is a preceding factor for the development of insulin resistance. In fact, it has already been reported that plasma glucose levels improve with administration of bezafibrate (4) and clofibrate (5) in type 2 diabetic patients, but the mechanism that triggers these improvements has yet to be fully clarified. To understand the relationship between insulin sensitivity and triglyceride metabolism, we examined 15 nonobese Japanese type 2 diabetic patients with hypertriglyceridemia (8 men and 7 women, aged 62.7 ± 2.0 years [mean ± SEM], BMI 23.7 ± 0.5 kg/m², fasting triglyceride levels >150 mg/dl) before and after treatment of bezafibrate for 2 months. There were no changes in diet and medication before and during the examination (12 patients were treated with glibenclamide, but the same doses were continued), except for the administration of 400 mg/day of bezafibrate. The statistical analysis was performed with the StatView 5 system (Statview, Berkeley, CA).

After the treatment, fasting triglyceride levels significantly fell from 256 ± 32 to 134 ± 13 mg/dl (P < 0.001). Fasting plasma glucose levels (162 ± 11 to 131 ± 7.0 mg/dl, P < 0.01) and fasting insulin levels (10.8 ± 1.1 to 8.4 ± 0.7 µU/ml, P < 0.05) also decreased significantly. Plasma HbA1c levels decreased, although not statistically significantly (7.6 ± 0.5 to 7.0 ± 0.3%, P = 0.19). To assess insulin sensitivity, we analyzed insulin resistance by using a homeostasis model assessment (HOMA-IR) (6), which provides a good correlation with an insulin sensitivity index derived from a minimal model approach as previously described (7,8). HOMA-IR levels significantly decreased from 4.1 ± 0.3 to 2.8 ± 0.3% after 2 months of therapy (P < 0.01). Bezafibrate is an agent known to increase the catabolism of very-low-density lipoproteins (9). Our present findings suggest that the improvement of triglyceride metabolism by bezafibrate lowers blood glucose levels by reducing insulin resistance and that elevated triglyceride levels are the preceding factors for the development of insulin resistance in nonobese Japanese type 2 diabetic patients.

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References
MTHFR Gene Polymorphism as an Exacerbation Factor of Diabetic Nephropathy in Type 2 Diabetes

Analysis in Japanese male hemodialysis patients

In 1998, diabetic nephropathy became the number one reason for the initiation of elective hemodialysis in Japan. However, its 5-year survival rate is still <50% because of both minor and major vascular complications. One of the exacerbation factors for the vascular endothelial cells is the level of homocysteine. Recently, C/T polymorphism at nucleotide position 677 of 5,10-methylenetetrahydrofolate reductase (MTHFR), the key enzyme in the remethylation pathway, was reported (1), and its variation was directly correlated to the blood level of homocysteine in hemodialysis (HD) patients (2). Although age dependency was previously ruled out (3), sex dependence of the MTHFR genotype distribution is not known. We first examined the genotype distribution in male maintenance HD patients (170 cases, age 61.0 ± 12.0 years [range 29–84], of which 73 were type 2 diabetic patients) compared with healthy male subjects (666 cases, age 56.6 ± 10.6 years [25–85]) and further analyzed type 2 diabetes in HD patients. Genotyping for MTHFR C/T polymorphism was performed on DNA extracted from peripheral blood of HD and control subjects with polymerase chain reaction (PCR)-restriction fragment–length polymorphism analysis with Hinf I digestion (1,3). Statistical analysis was performed with the $\chi^2$ test. Of the HD patients, the CC genotype was found in 56 patients (32.9%), and the CT + TT genotype was found in 114 patients (67.1%). In control subjects, the CC genotype was found in 339 patients (50.9%), and the CT + TT genotype was found in 327 patients (49.1%). The allele frequency of the 677T variant was significantly increased in the HD group compared with the control group (P < 0.0001); the odds ratio was 2.11 (95% CI 1.49–2.99). Of the type 2 diabetic subpopulation in HD patients, the CC genotype was found in 22 patients (30.1%), and the CT + TT genotype was found in 51 patients (69.9%). Type 2 diabetes in HD patients caused a statistically significant increase in the frequency of 677T (P = 0.0008); the odds ratio was 2.40 (1.44–4.00).

The recent interpretations of MTHFR gene polymorphism in type 1 versus type 2 diabetic nephropathy are controversial. The association of MTHFR gene polymorphism with diabetic nephropathy in type 2 diabetes was suggested by Neugebauer et al. (4). Smyth et al. (5), however, could not detect MTHFR gene polymorphism in type 1 diabetes. Because clinical nephrologists are often convinced that some patients with diabetic nephropathy should drop into elective hemodialysis faster than others, even though they may be under the same clinical management for blood pressure, blood glucose level, etc., we attained the records of type 2 diabetic patients on HD (medical records of the onset of proteinuria were available for 26 of 73 type 2 diabetic patients) to analyze retrospectively C/T polymorphism and the duration between the onset of proteinuria and the initiation of elective hemodialysis. Statistical analysis was performed by using the Kaplan-Meier test and the log-rank test, and the level of significance was set at P < 0.05. The data demonstrated a statistically significant correlation between the presence of 677T and the progression of renal failure. This observation suggests that the MTHFR gene may be an aggravating factor of diabetic nephropathy.

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**References**


**Contribution of Abnormalities of Thyroid Hormones to Type 2 Diabetes**

Although an enormous amount of data concerning the etiologic role of insulin in hyperglycemia has been accumulated over the last decades (1–4), there are still unrealized missing links between the role of insulin and glucose needed to achieve further insights into the pathogenic mechanism of type 2 diabetes. Available evidence suggests that glucose homeostasis appears to be the result of the T3 and insulin synergistic regulation of gene transcriptions involved in metabolic pathways of glucose and lipids (2,5). Specifically, authors are surprised that T3 regulates a wide range of gene expression of glucose transporters, which include all of the regulatory enzymes essential for oxidation of glucose and lipids, glucose storage, glycolysis, cholesterol synthesis, cholesterol transfer, and glucose-lipid metabolic pathways. Most importantly, there is a strong indication that T3 also regulates insulin secretion via transcription of glucokinase and ATP-sensitive K+ and Ca2+ channels of the pancreas (5).

The diagram, which mapped the expande regulatory range of T3 and insulin on gene expression covering all the regulatory proteins required for the intermediary metabolic pathways of glucose and lipids, was completed to delineate the regulatory sites of T3 and insulin. This diagram covers every step of Randle's glucose-lipid cycle.
cycle, from the glucose uptakes via glut-4 of the plasma membrane to the post-insulin receptor phase in muscle and adipose tissue (figure not shown).

Interestingly, the list of these regulatory proteins, which are under the regulation of both T3 and insulin, also encompasses all of the presently known candidate genes (6), which indicates that the reduced expression of these genes, due to the abnormal levels of T3 and/or insulin, mimics the same disorders characteristic of diabetes, which are the result of defective candidate genes.

Thus, it is plausible to assume that abnormalities of T3 and/or insulin are biologically responsible for whole ranges of abnormalities in enzymes involved in the metabolic pathways of glucose and lipids precipitating the disorders characteristic of diabetes, (insulin resistance, hyperinsulinemia, hypertriglyceridemias, central obesity and hyperglycemia) via disturbing the transcription of the enzymes required for intermediary metabolites needed for glucose-lipid pathways.

Furthermore, documented evidence of the influence of T3 on glucose levels was supported and strengthened by a study of the effects of T3 on the glucose levels of 107 African-American women from the Virgin Islands aged >45 years. The study indicated that T3 levels <100 ng/dl were associated with an odds ratio of 11.87 (95% CI 5.63–39.27) when compared with control subjects (whose mean T3 levels were 111.87. Moreover, logistic regression of T3 levels, when adjusted for triglyceride levels and waist-circumference measurements, lowered the risks of diabetes from 5.38 (2.01–12.05) to 3.79 (1.19–12.09) in the test subjects, and from 3.14 (1.71–3.42) to 2.41 (127–4.60) in the control subjects. Thus, these results are consistent with the biological role of T3, which regulates the levels of glucose and lipids and supports the present hypothesis of the cooperative contribution of T3 and insulin to homeostasis of glucose and lipids, thereby type 2 diabetes. The mean T3 level of the test subjects (84.24 ng/dl) was significantly lower (P < 0.001) than that of the control subjects (110.14 ng/dl).

We believe that the synergistic influence of T3 and insulin on gene expression of enzymes for glucose-lipid cycles and cholesterol-related metabolic pathways is a missing link that may offer further insights into understanding diabetes.

Therefore, in an effort to gain a better understanding of the pathogenesis of diabetes, we are proposing the integration of a model that will allow better insight into the manifold influences of T3 and insulin on homeostasis of glucose and lipids.

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