Hyperglycemia Is a Factor for an Increase in Serum Ceruloplasmin in Type 2 Diabetes

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OBJECTIVE — To examine if there is a correlation between high blood glucose and serum ceruloplasmin (Cp) levels.

RESEARCH DESIGN AND METHODS — Serum Cp levels were measured in 637 patients with type 2 diabetes (all type 2 diabetes group). For the follow-up type 2 diabetes group, 161 patients who had not had any changes in their situation during the last year that are known to influence serum Cp levels were reexamined 1 year later. The control group was composed of 158 healthy individuals. Serum Cp and blood HbA_{1c} levels were measured by radial immunodiffusion and high-performance liquid chromatography assays, respectively.

RESULTS — Serum Cp levels in the all type 2 diabetes group were significantly higher than those in the control group ($P < 0.0001$), although the serum Cp levels did not correlate with the blood HbA_{1c} levels in the all type 2 diabetes group ($r = 0.055$, $P = 0.351$). Then we evaluated those factors (Δ-log Cp and Δ-HbA_{1c}) in the follow-up type 2 diabetes group to minimize changes from the genetic differences and to exclude any known factors influencing serum Cp levels. This indicated that the Δ-HbA_{1c} had a positive correlation to the Δ-log Cp ($r = 0.304$, $P < 0.0001$).

CONCLUSIONS — A persistent high blood glucose (namely HbA_{1c}) is associated with an increase in serum Cp levels over 1 year.

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Ceruloplasmin (Cp) is a circulating blue multicopper oxidase that contains >95% of copper in the plasma. Cp is synthesized mainly in the liver as a single-chain polypeptide, and after the incorporation of six atoms of copper early in the biosynthetic pathway, it is secreted into the plasma as an α2-glycoprotein (1,2). Although its precise biological roles are unknown, it may be related to angiogenesis (3), copper transport (4), iron metabolism (5), and antioxidant defense (6). Serum level of Cp increases during infection and tissue injury (7). On the other hand, a decrease of the protein in the plasma is observed in some diseases, such as Wilson's disease (8), hereditary hypoceruloplasminemia (9), and hereditary ceruloplasmin deficiency (HCD). HCD is an autosomal recessive disease characterized by neurological abnormalities such as progressive cerebral degeneration, complete Cp deficiency, and excessive storage of iron in the systemic organs, such as liver and brain (10). In many HCD cases, type 2 diabetes is the first symptom, and 5–20 years later at ages 40–60 years, the neurological abnormalities occur (10).

An increase in serum Cp levels has also been reported in type 2 diabetes (11–14). However, it has been reported that blood HbA_{1c} levels, duration of type 2 diabetes, patient age, and the presence or absence of diabetes complications are not major factors influencing its increase (11–15). So, the question is, what factor increases serum Cp in type 2 diabetes? To clarify it, we measured serum Cp levels from a relatively large number of type 2 diabetes patients and confirmed an increase in serum Cp levels in type 2 diabetes. Next, we tried to find what factors cause the increase in this large type 2 diabetes population and found that a high blood glucose level itself is such a factor.

RESEARCH DESIGN AND METHODS

Subjects

In February 1996, serum levels of Cp and copper (Cu) were measured in all 637 outpatients with type 2 diabetes in our university hospital (320 cases) and its affiliated hospital (317 cases). This group was designated as the all type 2 diabetes group. Age, duration of illness, sex, and blood levels of HbA_{1c} were monitored in all patients in our university hospital and some in the affiliated hospital. One year later (February 1997), we reexamined Cp and HbA_{1c} levels in 161 type 2 diabetes patients in our university hospital (follow-up type 2 diabetes group). The remaining 159 patients in our hospital were excluded because they either did not come (85 cases) or had additional diseases (74 cases) that are known to increase serum Cp (inflammatory diseases, collagen diseases, or malignancy) or to decrease it (malnutrition, liver cirrhosis, severe nephrotic syndrome, or HCD). The diagnoses of these diseases mentioned above were made from both clinical symptoms and ordinary laboratory and radiological examinations obtained during the follow-up period. Some of the patients had long-standing history of such diseases even before the beginning of this project. Use of estrogen, as in the oral contraceptive pill, is also known to increase serum Cp (16). How-

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Abbreviations: Cp, ceruloplasmin; HCD, hereditary ceruloplasmin deficiency.
A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of each study group*

Study group	Number of individuals	Sex (W/M)	Age (years)	Cp	Cu	Duration of diabetes	HbA_{1c} (%)
All type 2 diabetes	637	184/190*	61.4 ± 12.6†	1.467 ± 0.085†	96.97 ± 18.5	ND	6.6 ± 1.3*
Follow-up type 2 diabetes	161	78/83	64.3 ± 10.0†	1.463 ± 0.071†	96.78 ± 15.6	10.15 ± 7.1	6.6 ± 1.2
Control	158	106/52	39.5 ± 14.8	1.418 ± 0.078	ND	None	ND; normal, 3.8–5.4

Data are *n* or means ± SD. *Sex and blood HbA_{1c} levels were monitored only in 374 and 320 individuals, respectively, in the all type 2 diabetes group. †Statistical significance (<0.05) to the normal value. Serum Cp levels were expressed in logarithmic values (log_{10} of each value in milligrams per deciliter). Serum Cu levels were expressed in micrograms per deciliter. ND, not determined.

ever, no one in our study group was reported to be taking these drugs. Changes of serum Cp and blood HbA_{1c} levels in this interval were shown as Δ-log Cp and Δ-HbA_{1c}, respectively. The patients with Δ-HbA_{1c} of less than −1%, between −1% and 1%, and more than 1% were grouped as groups less than −1, −1 to 1, and greater than 1 (the numbers of individuals in each group were 8, 130, and 23), respectively. The follow-up type 2 diabetes group was subdivided into groups 0, 1, 2, and 3 according to the number of diabetic complications a patient had (the numbers of individuals in each group were 68, 57, 20, and 7, respectively). Nine patients in the group were excluded because no information about their diabetic complications was available. In this study, we counted only the number of diabetic complications (retinopathy, nephropathy, and neuropathy) and did not consider the severity of the complications. Diabetic retinopathy was evaluated by specialized ophthalmologists. Nephropathy was diagnosed when patients showed proteinuria and/or microalbuminuria (>30 μg/ml). Neuropathy was diagnosed by the presence of abnormal sensation in the extremities and/or 45 m/s or less in motor nerve conduction velocity of the median nerve. This project was approved by the Ethical Committee of Yamagata University Hospital, and informed consent was obtained from each participant at the beginning of the project.

Serum Cp levels of 158 healthy individuals (control group) were also examined. The details of these study groups are summarized in Table 1. The control individuals were recruited from a factory, and the mean age of the control group (39.6 ± 14.8 years) was lower than the mean ages of the type 2 diabetes groups (all: 61.4 ± 12.6 years; follow-up: 64.3 ± 10.0 years).

Assay

Serum levels of Cp and Cu were measured at a commercial laboratory, Special Reference Laboratory (SRL, Tokyo, Japan). Blood levels of HbA_{1c} were determined at the central laboratory of our university hospital. In brief, serum Cp was assayed by radial immunodiffusion assay using Boehringer nephelometer analyzer (Boehringer, Germany). Blood, which was used for serum Cu assay by atomic absorption spectrometry, was collected in an acid-washed plastic conical tube to avoid contamination of trace amount of metals from the tube itself. Blood HbA_{1c} levels were measured by high-performance liquid chromatography assay (Hi-Auto A1c HA8121; Kyoto Dai-ichi Kagaku, Japan). The intra- and interassay coefficients of variation (%) for the assays of serum Cp and blood HbA_{1c} were 2.54 and 1.43 and 0.49 and 0.55, respectively.

Statistical analysis

The distribution of normal serum Cp levels was log normal. Therefore, measured values were transformed to their logarithms before calculating the SD score to obtain age-independent values for comparison. Data are given as means ± SD. The statistical significance of differences between two groups and three or more groups were determined by the Student's *t* test and analysis of variance, respectively. For analysis of variance, Fisher's PLSD test was used as post hoc test. Correlations between variables were assessed using univariate linear regression analysis. $P < 0.05$ was accepted as statistically significant.

RESULTS

Serum Cp levels of the patients in the all type 2 diabetes group

The serum Cp levels of patients in the all type 2 diabetes group were significantly higher than those of the control group ($P < 0.0001$) (Fig. 1*A*). However, the serum Cp levels in

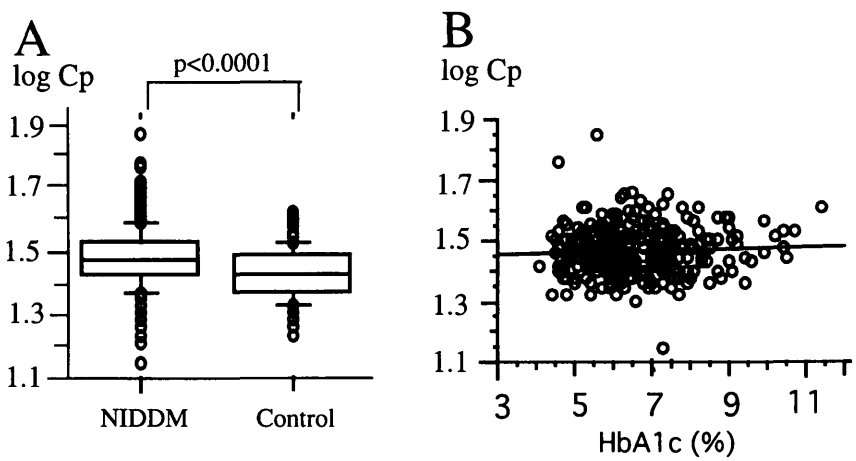

Figure 1—*Serum Cp levels in type 2 diabetes. The levels were expressed in logarithmic values* (log_{10} *of each value in milligrams per deciliter). The box and whisker plots of the serum Cp levels are shown in A. The five horizontal lines in the box plots (from the bottom to the top) indicate 10th, 25th, 50th (median), 75th, and 90th percentiles. Cp levels below the 10th or above the 90th percentiles are indicated as open circles. The serum Cp levels are higher in the all type 2 diabetes group* (n = 637) *than in the control group* (n = 158) ($P < 0.0001$). *In B, no significant correlation is seen between serum Cp and blood* HbA_{1c} *levels* ($P = 0.351$). *The best-fit equation for the regression is log Cp = 1.444 + 0.003 ×* HbA_{1c}. $r = 0.055$, $P = 0.351$.

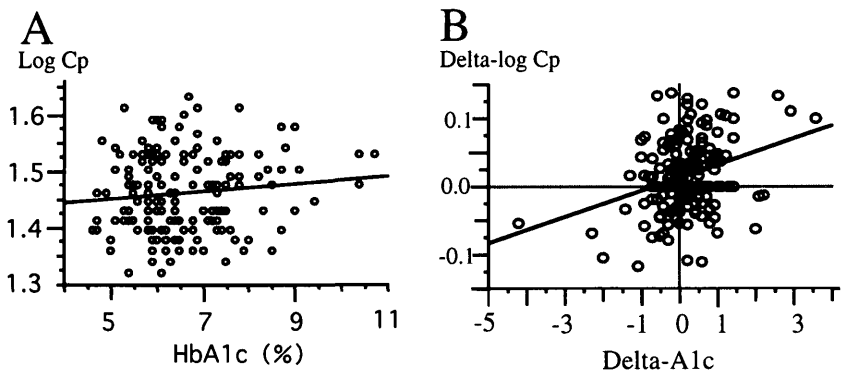

Figure 2—*Blood glucose levels and serum Cp levels in the follow-up type 2 diabetes group. No significant correlation is observed between the serum Cp levels and blood HbA_{1c} levels (A; P = 0.1504). The changes at 1 year in the serum levels of Cp expressed in log and in the blood levels of HbA_{1c} (%) are designated as Δ-log Cp and Δ-HbA_{1c}, respectively. The Δ-log Cp is correlated significantly with the Δ-HbA_{1c} (B; P < 0.0001). The best-fit equations for each regression are as follows: A: Log Cp = 1.418 + 0.007 × HbA_{1c}. r = 0.114, P = 0.1504. B: Δ-log Cp = 0.012 + 0.019 × Δ-HbA_{1c}. r = 0.304, P < 0.0001.*

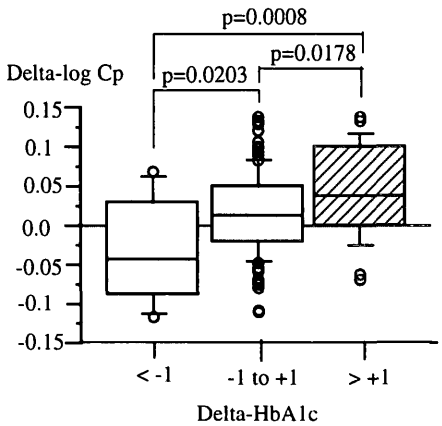

Figure 3—*Box and whisker plots analysis in the follow-up type 2 diabetes group. The serum Cp levels in the groups with Δ-HbA_{1c} <−1 (□), −1 to 1 (□), and >1 (▨) (n = 8, 130, and 23) are shown. The bars represent the SD.*

the all type 2 diabetes group did not significantly correlate with the blood HbA_{1c} levels (r = 0.055, P = 0.351) (Fig. 1*B*). The serum Cp levels showed a very high correlation with the serum Cu levels (log CP = 1.142 + 0.003 × Cu (μg/dl); r = 0.727, P < 0.0001) (data not shown).

Relationship between serum Cp levels and blood glucose levels in the follow-up type 2 diabetes group

To adjust for genetic background, we evaluated serum Cp and HbA_{1c} levels from the same people after an interval of 1 year. The levels of serum Cp and blood HbA_{1c} in this follow-up type 2 diabetes group were similar to those of the all type 2 diabetes group (P < 0.0001). Moreover, the serum Cp levels did not show a significant correlation with the blood HbA_{1c} levels (r = 0.114, P = 0.150) (Fig. 2*A*). But Δ-log Cp did show a significant positive correlation with Δ-HbA_{1c} (r = 0.304, P < 0.0001) (Fig. 2*B*). There seem to be several outlying points that may have some effect on the significance of the relationship. However, correlation was still observed (P = 0.0007), even when two such points with Δ-HbA_{1c} levels of −4.2 and 3.6 were removed from the data. To confirm this positive correlation in another way, we divided the follow-up type 2 diabetes group into three groups (groups less than −1, from −1 to 1, and greater than 1) on the basis of their Δ-HbA_{1c}. As shown in Fig. 3, the Δ-log Cp was increased with increasing levels of HbA_{1c} (P = 0.0026). The difference of Δ-log Cp was especially marked between the groups −1 and 1 (P = 0.0008).

Factors that might affect the serum Cp levels

No positive correlation was observed between serum Cp levels and the number of diabetes complications (P = 0.1516) (Fig. 4). However, the patients with no diabetic complications had significantly lower serum Cp levels than did those with one complication (P = 0.0300) (Fig. 4). Also revealed was that even the diabetes patients with no complications (group 0) had significantly higher serum Cp levels (1.449 ± 0.071) than did control subjects (P = 0.0009). The age and the duration of type 2 diabetes showed no significant correlation with serum Cp levels (data not shown).

CONCLUSIONS — The previous studies with a relatively small number of patients demonstrated that serum Cp levels were higher in diabetic than in normal individuals (11,12). We confirmed this with a larger number of diabetes patients (637 individuals). The question is, what increases the serum Cp level in diabetes? We searched for many factors that may be associated with diabetes. As mentioned in RESULTS, serum Cp levels were highly correlated with serum Cu levels. Therefore, we measured only serum Cp levels for further study. Serum Cp has been reported to be increased or decreased in several diseases. Its increase is observed in some inflammatory diseases, such as tuberculosis and acute upper respiratory infection; some collagen diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and rheumatic fever; malignant tumors; obstruction of bile duct; anemia; and administration of estrogen (17). Its decrease is noted in Wilson's disease, Menkes' syndrome, HCD, liver failure, protein losing gastroenteropathy, and severe nephrotic syndrome (17). A simple explanation for Cp increase is that Cp is an acute-phase protein, although the details of the mechanism are unclear. A decrease in serum Cp can be explained by an impairment of Cp production and/or losing of Cp from the serum. Here, we aimed at finding isolated factors associated with an increase in serum Cp levels in type 2 diabetes.

Several factors have been evaluated to see if they have a correlation with serum Cp levels. The presence of diabetes complica-

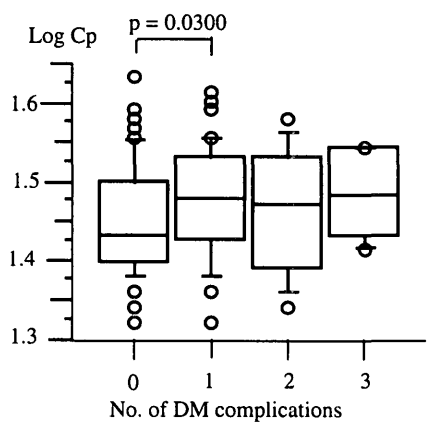

Figure 4—*Factors that may affect serum Cp levels. This study was done with the follow-up type 2 diabetes group. The patients with 0, 1, 2, and 3 diabetic complications were subdivided into groups 0, 1, 2, and 3 (n = 68, 57, 20, and 6), respectively. Although the number of diabetic complications does not correlate significantly with serum Cp levels (P = 0.1516), there is a significant correlation of serum Cp levels between groups 0 and 1 in the type 2 diabetes group (P = 0.0300).*

tions has been reported as such a factor (13,14). Besides that, it was reported that neither blood HbA_{1c} level, patient age, nor duration of type 2 diabetes was significantly correlated with serum Cp levels (11–15,18,19). But their results were derived from a relatively small number of patients. In the present study using a much larger population of type 2 diabetes patients, we tried to see if there is a correlation between those factors and serum Cp levels. We obtained the result that none of these factors was simply correlated with an increase in serum Cp levels. Nevertheless, we could not so easily rule out the possibility that there is a correlation between blood glucose and serum Cp, because a high blood glucose level is the fundamental factor in type 2 diabetes. So, we explored whether there was a correlation between the changes in blood glucose and serum Cp levels after adjusting for genetic background by checking those factors after a 1-year interval and excluding from the study group (the follow-up type 2 diabetes group) patients who had any factors known to change serum Cp levels during this 1-year interval. The values after adjustment were designated as Δ-log Cp and Δ-HbA_{1c}. Indeed, the Δ-log Cp showed a significant correlation with the Δ-HbA_{1c} ($r = 0.304$, $P < 0.0001$). This was confirmed by another analysis (Fig. 3). The serum Cp levels of the patients in the greater than 1 follow-up type 2 diabetes group were significantly higher than those in the less than −1 group ($P = 0.0065$). These results strongly indicate that high blood glucose is associated with an increase in serum Cp levels in type 2 diabetes.

An increase in serum Cp in type 2 diabetes could generate excess oxidized LDL, which causes atherosclerosis (20). It could also cause vascular injury by generating free radicals, such as hydrogen peroxide, in the course of oxidization of serum homocysteine (21). Alternatively, Cp is thought to be a scavenger. Its increase could be explained by an increase in oxidative stress in type 2 diabetes (17,18).

In conclusion, a high blood glucose level may cause an increase in serum Cp in type 2 diabetes, which could be associated with the development of vascular injury (diabetic complication) in type 2 diabetes.

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