centrifugal analyzer (10). In the physiologic range, the fluorometric assay correlated with the colorimetric assay, but the high specific activities obtained with the 4-MUF substrate yielded AFU values threefold higher than those obtained with the 4-NPF substrate. The fluorometric detection also yielded a good accuracy without the interferences commonly observed with colorimetric determinations. The micromethod we describe can be applied to other discrete automated fluorometric analyzers and fluorescence microplate automated systems.

In this study, we confirm the existence of a plasma AFU activity polymorphism with a trimodal distribution (9, 11). Median values calculated for low (variant), medium, and high activities were close to those obtained by Wood (9) from a healthy student population. However, because of the strict selection of the French adult population studied, the upper limit of “normal” values in the present study was 1.4-fold lower. The anonymous collection of plasma did not allow us to study age or gender as variables. However, previous studies (6, 12) detected no gender differences or significant age variations in subjects >25 years of age.

We conclude that automation of the fluorometric AFU method greatly improved sensitivity, specificity, and practicability of the assay. This test, therefore, can be used in large-scale screening for fucosidosis where values are lower than the low variant (13). However, the trimodal distribution of plasma AFU activity limits the diagnostic value of this test for acquired diseases. Nevertheless, as suggested by Giardina et al. (3), determination of plasma AFU activity may be very useful in the follow-up of cirrhotic patients for the early detection of hepatocellular carcinoma.


Eiberg H, Mohr J, Nielsen S. Linkage of plasma catalase activities and diabetes mellitus. The free radicals that damage cellular macromolecules are scavenged by a range of antioxidant enzymes. Superoxide dismutase catalyzes the conversion of superoxide anion into hydrogen peroxide, and catalase inherited as an autosomal recessive trait, and in hypocatalasemia, heterozygosity of the acatalasemia gene, the defense system against hydrogen peroxide is diminished; however, no biochemical changes have been reported for this syndrome (6, 7). Furthermore, recent findings concerning the connection of decreased antioxidant enzyme activities and diabetes mellitus lack clarification of the mechanism (8, 9).

We have reported on two acatalasemic sisters in the first Hungarian acatalasemic family (10) and nine hypocatalasemic families (11) with 37 hypocatalasemics. The frequencies for acatalasemia and hypocatalasemia in Hungary are 0.05 in 1000 and 1.8 in 1000.

References
We describe here the first comprehensive study of the biochemical markers of lipid and carbohydrate metabolism in acatalasemia and hypocatalasemia. We studied one acatalasemic and five hypocatalasemic Hungarian families with 2 acatalasemic females, 28 hypocatalasemic family members (15 females and 13 males), and 28 normocatalasemic family members (15 males and 13 females).

Serum glucose was determined by a glucose oxidase-peroxidase method (glucose test; Reanal), serum fructosamine (Roche Fructosamine Test; Hoffmann-La Roche), blood hemoglobin A1C (DIAMAT; Bio-Rad), triacylglycerides (triglyceride GPO-PAP Test; Boehringer Mannheim), choles terol (cholesterol CHOD-PAP Test; Boehringer Mannheim) on a Boehringer/Hitachi 717 analyzer. LDL-cholesterol was calculated according to the Friedewald formula. Serum apolipoprotein (Apo) A1, Apo B, and lipoprotein(a) [Lp(a)] were measured on a COBAS MIRA analyzer (Hoffmann-La Roche). Oxidative modification of LDL was measured by a microassay of the oxidative resistance of LDL based on the hemin-catalyzed oxidation of LDL (12).

Blood catalase activity was determined spectrophotometrically (10, 11), with a reference mean ± SD of 113.3 ± 16.5 MU/L (n = 1756). We selected a normocatalasemic family member age-matched to each hypocatalasemic family member. The Student t-test was used to evaluate the statistical significance of difference between the two groups.

The results are shown in Table 1. The increased glucose, hemoglobin A1C, and fructosamine in the patients reflected the higher incidence of diabetes in the affected members (n = 8) than in controls (n = 0). Seven of the affected members had type 2 diabetes, and one had type 1. Although decreased blood catalase activity in type 1 and type 2 diabetes mellitus has been reported (8, 9, 13–15), the high frequency (23%) of diabetes mellitus we found in hypocatalasemic and acatalasemic patients is a new finding. The toxic effect of increased hydrogen peroxide concentrations on either the pancreatic cells or the peripheral tissues may be involved in the pathogenesis of the disease. A study using pancreatic insulin-producing cells from rats showed that catalase plays a critical importance for the removal of reactive oxygen species (16).

Significant (P <0.048) changes were detected in cholesterol, LDL-cholesterol, Apo A1, Lp(a), and Apo B concentrations, and LDL oxidative resistance. These values in the diabetic patients did not show a significant (P >0.88) change when compared with those of the control group (cholesterol, 4.79 ± 1.13 vs 4.56 ± 0.89 mmol/L; LDL-cholesterol, 3.04 ± 0.38 vs 2.83 ± 0.97 mmol/L; Apo A1, 1.55 ± 0.29 vs 1.51 ± 0.38 g/L; Apo B, 1.09 ± 0.16 vs 1.05 ± 0.23 g/L; Lp(a), 259 ± 144 vs 212.1 ± 193.8 mg/L). These data suggest that the hypocatalasemia is the main contributor of the lipid abnormalities. The triglyceride and HDL-cholesterol concentrations were similar in the two groups.

In the two acatalasemics (both diabetic), increases (compared with their age- and gender-matched pairs) were also seen in cholesterol (6.39 vs 4.18 mmol/L), LDL-cholesterol (4.14 vs 2.46 mmol/L), Apo A1 (1.69 vs 1.53 g/L), Apo B (1.35 vs 1.10 g/L), and Lp(a) (294 vs 222 mg/L), with lower LDL oxidative resistance (2580 vs 4480 s) and increased glucose (8.6 vs 5.4 mmol/L), fructosamine (256 vs 210 μmol/L), and hemoglobin A1C (7.1% vs 4.5%). Changes in cholesterol, LDL-cholesterol, Apo A1, Apo B, Lp(a), and LDL oxidative resistance have not been reported for acatalasemic and hypocatalasemic patients and could be attributed to the increased oxidation of cholesterol, especially of LDL-cholesterol. The connection between lipid peroxidation and catalase activity has been reported in other diseases (4, 9, 13, 14). The change in conventional (cholesterol, LDL-cholesterol, and Apo B) and in nonconventional [Lp(a), LDL oxidative resistance] risks may mean a higher risk for these patients.

In addition to the condition itself, hypocatalasemia is seen with increased frequency in other disorders [e.g., anemia, tumors, schizophrenia, and atherosclerosis (4)], yielding a prevalence of ~1%. We conclude that these acatalasemic and hypocatalasemic subjects are at increased risk of diabetes mellitus and atherosclerosis.

Table 1. Markers (mean ± SD) of carbohydrate and lipoprotein metabolism in five hypocatalasemic families in Hungary.

<table>
<thead>
<tr>
<th>Analyte or condition</th>
<th>Hypocatalasemia (n = 28)</th>
<th>Controls (n = 28)</th>
<th>P</th>
<th>Reference values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>5.40 ± 1.02</td>
<td>5.10 ± 0.69</td>
<td>0.193</td>
<td>3.6–6.0</td>
</tr>
<tr>
<td>Fructosamine, μmol/L</td>
<td>216.1 ± 28.2</td>
<td>214.0 ± 17.1</td>
<td>0.705</td>
<td>&lt; 285</td>
</tr>
<tr>
<td>Hemoglobin A1C, %</td>
<td>5.40 ± 1.01</td>
<td>5.10 ± 0.69</td>
<td>0.225</td>
<td>4.2–6.1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>17.8%</td>
<td>0%</td>
<td>&lt; 0.001</td>
<td>2–4%</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.35 ± 1.28</td>
<td>2.24 ± 1.26</td>
<td>0.758</td>
<td>&lt; 1.7</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.58 ± 0.97</td>
<td>4.56 ± 0.89</td>
<td>0.001</td>
<td>&lt; 5.2</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>3.44 ± 0.96</td>
<td>2.83 ± 0.97</td>
<td>0.003</td>
<td>&lt; 3.4</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.42 ± 0.30</td>
<td>1.38 ± 0.39</td>
<td>0.664</td>
<td>&gt; 1.2</td>
</tr>
<tr>
<td>Apo A1, g/L</td>
<td>1.76 ± 0.10</td>
<td>1.51 ± 0.38</td>
<td>0.003</td>
<td>&lt; 1.15</td>
</tr>
<tr>
<td>Apo B, g/L</td>
<td>1.29 ± 0.25</td>
<td>1.05 ± 0.23</td>
<td>0.001</td>
<td>&lt; 1.0</td>
</tr>
<tr>
<td>Lp(a), mg/L</td>
<td>368.7 ± 214.0</td>
<td>212.1 ± 193.8</td>
<td>0.048</td>
<td>&lt; 300</td>
</tr>
<tr>
<td>LDL oxidative resistance, s</td>
<td>4130 ± 1467&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5441 ± 1828&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Catalase, MU/L</td>
<td>48.9 ± 15.8</td>
<td>106.3 ± 18.5</td>
<td>&lt; 0.001</td>
<td>80.3–146.3</td>
</tr>
<tr>
<td>Age, year</td>
<td>44.7 ± 19.9</td>
<td>42.7 ± 17.8</td>
<td>0.778</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>n = 18.
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References

Effect of Prolonged Storage on the Activities of Superoxide Dismutase, Glutathione Reductase, and Glutathione Peroxidase, Clifford Abiaka,1* Farida Al-Awadi,2 and Samuel Olusi3 (1 Department of Medical Laboratory Science, Faculty of Allied Health Sciences, Kuwait University, P.O. Box 31470, Kuwait; Departments of 2 Biochemistry and 3 Pathology, Faculty of Medicine, Kuwait University, Kuwait; * author for correspondence: fax 965-4830937, e-mail clifford@hsc.kuniv.edu.kw)

Reactive oxygen species such as superoxide and hydrogen peroxide react in human tissues to form hydroxyl free radicals, especially when catalyzed by transition metals, e.g., iron [Fe(II)] and copper [Cu(I)]. The product is highly electrophilic and damaging to surrounding tissues and is implicated in the pathology of debilitating human diseases such as atherosclerosis (1), Parkinson disease and other neurodegenerative disorders (2), and cancer (3). Copper and zinc-superoxide dismutase (CuZn-SOD; EC 1.15.1.1), glutathione peroxidase (GPX; EC 1.11.1.9), and glutathione reductase (GR; EC 1.6.4.2) are cellular antioxidants that can protect cells from the potentially harmful effects of reactive oxygen species (4–6). Assays of the activities of these mainly intracellular enzymes form part of the indirect determination of the activity of free radicals.

Measurements of erythrocyte CuZn-SOD, GPX, and GR usually are performed in the assessment of antioxidant status, and there has been speculation on the stability of these enzymes over a long period of storage. In this study, we investigated the long-term stability of CuZn-SOD, GPX, and GR in washed erythrocyte hemolysates by comparing the activities in stored hemolysates with those in hemolysates from freshly drawn blood. Subjects were recruited from blood donors (n = 24) with the consent of the ethics committee. The blood donors fulfilled the health requirements of the blood transfusion center, i.e., absence of contagious disease, medication, or history of viral hepatitis, malaria, and drug addiction. Venous blood (5 mL) was collected from each donor into a potassium-EDTA Vacutainer Tube by means of a cannula inserted into the arm of the recumbent donor. The blood was centrifuged within 4 h of sampling at 1620g for 10 min in a refrigerated centrifuge at 4 °C to separate the plasma. The buffy coat was removed, and the remaining erythrocytes were drawn from the bottom, washed three times in cold saline (9.0 g/L NaCl), and hemolyzed by the addition of an equal volume of ice-cold demineralized ultrapure water (MilliQ plus reagent grade; Millipore) to yield a 50% hemolysate. Aliquots of the hemolysates (500 μL) were prepared for immediate assay of CuZn-SOD, GPX, and GR. Additional aliquots (500 μL) of the remaining hemolysates were dispensed into 1.5-mL capacity Beckman microfuge tubes (Beckman Instruments), capped, and frozen at −80 °C; these aliquots were assayed for CuZn-SOD, GPX, and GR after storage for 5, 12, and 21 months. To the 50% hemolysates (500 μL), was added 1 mL of demineralized water; 10 μL of each diluted hemolysate was brought to a final volume of 1.25 mL with Ransod sample diluent (a solution of 0.01 mol/L phosphate buffer, pH 7.0), thus bringing the final dilution of the hemolysates assayed for the activity of CuZn-SOD to 1.500. This dilution gives a 40–60% inhibition by hemolysate CuZn-SOD of the generation of superoxide anion free radical (O2•−) by the reagent xanthine and xanthine oxidase (XOD).

Measurement of CuZn-SOD activity was performed using Ransod reagents (Randox Laboratories) and is based on the method developed by McCord and Fridovich (7) coupling O2•− generators (xanthine and XOD) with an O2•− detector [2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride]. Absorbance was monitored in a Beckman DU 7500 spectrophotometer (Beckman Instruments, Fullerton, California).