exercise the blood pressure cuff was deflated to reestablish circulation. The results of two subjects are shown in Table 1. Both are normal responses in that the basal lactate was <2 mmol/L and that this was followed by an increase to >2.5 mmol/L in the first 4–5 min post exercise, the peak being between 1 and 3 min.

The ischemic exercise test is not a frequently performed test, and the procedure for it is not described in easily available clinical chemistry textbooks, e.g., *Tietz Textbook of Clinical Chemistry* (1999) and Kaplan and Pesce's *Clinical Chemistry* (1989), or in *The Metabolic and Molecular Bases of Inherited Disease (1)*. This makes it all the more important to emphasize that to avoid false-positive results and unnecessary traumatic repetitions of the test, the blood pressure cuff must be deflated before the postexercise specimens are taken.

Since performing this study, we have discovered that the procedure for the ischemic exercise test is correctly described by Thomas (3). However, his prescription of ischemic exercise for 2 min (rather than 1 min) can be difficult for many people and appears to be unnecessary.

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A Simple PCR-Heteroduplex Screening Method for Detection of a Common Mutation of the Catalase Gene in Hungary, *László Góth,[1*] András Gorzsás,[1] and Tibor Kalmár[2]* ([1] Department of Clinical Biochemistry and Molecular Pathology, Medical School, University of Debrecen, PO Box 40, H-4012 Debrecen, Hungary; [2] Department of Genetics, Biological Research Institute, PO Box 521, H-6701 Szeged, Hungary; * author for correspondence: fax 36-52-417-631, e-mail goth@jaguar.dote.hu)

The enzyme catalase (EC 1.11.1.6) has a predominant role in controlling the concentration of hydrogen peroxide in human erythrocytes (1). Hydrogen peroxide is involved in physiological processes, but its increased concentration may contribute to the pathogenesis of various diseases, such as diabetes and atherosclerosis. Human erythrocytes with high catalase content provide a general defense against toxic concentrations of hydrogen peroxide (2, 3). Hypocatalasemia is the heterozygous state of the acatalasemia gene and is inherited as an autosomal, recessive trait without any characteristic clinical sign. The frequency of hypocatalasemia in East Asia is 0.2–0.4%, whereas in two Iranian populations it is 0.5% (4, 5). There are only limited data available on the disease-causing mutations. The splicing mutation (guanine-to-adenine substitution) at the fifth position of intron 4 and the 358T

deletion in exon 4 have been detected in five Japanese patients (6–8).

We have reported on nine hypocatalasemic families for the first time in Hungary (9). The frequency of inherited hypocatalasemia is 0.18% in Hungary (9). The syndrome-causing mutations detected in Japanese patients (6–8) have not been found in the Hungarian hypocatalasemic patients (10, 11).

We report here on a new catalase mutation that caused hypocatalasemia in three (M, D, and G) Hungarian hypocatalasemic families. For this mutation, we amplified all exons and exon-intron junctions of the catalase gene by PCR. These PCR products were screened for mutations by a simple heteroduplex detection method. The mutation was determined by nucleotide sequence analysis.

Genomic DNA was isolated from 23 hypocatalasemic and 25 normocatalasemic members of six Hungarian hypocatalasemic families. The DNA extraction was made by a QIAamp Blood Kit (QIAGEN). The PCR amplification was performed in a total volume of 10.5 μL, containing 1 μL of genomic DNA (0.2 μg/μL), 1.6 μL of four dNTPs (1.25 mmol/L each), 1 μL of each primer (10 μmol/L), 0.5 μL of 5 U/μL Taq polymerase, 1 μL of 8.3 mmol/L MgCl$_2$, and 1 μL of buffer. PCR reagents were purchased from Pharmacia. Thirty cycles of amplification at 94, 55, and 72 °C for 0.5, 0.5, and 1 min, respectively, were performed in a DNA thermal cycler (TC 1; Perkin-Elmer Cetus). Oligonucleotide primers were synthesized by Pharmacia, according to the sequences reported by Kishimoto et al. (7).

Heteroduplex analysis was performed according to the Hydrolink protocol (AT Biochem). PCR product (2 μL) was heated to 94 °C, cooled down slowly, and then loaded onto a Hydrolink gel (280 × 180 × 0.75 mm). DNA bands
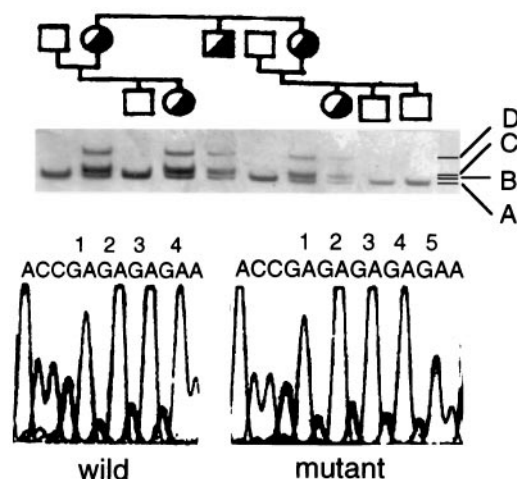

Fig. 1. Pedigree (*top*), heteroduplex pattern (*middle*), and nucleotide sequence analysis (*bottom*) of hypocatalasemic family G.

(*Top*), ◐, hypocatalasemic female; ◪, hypocatalasemic male; □, normocatalasemic male. (*Middle*), the heteroduplex pattern *A* represents a wild-wild homoduplex, *B* represents a mutant-mutant homoduplex, and *C* and *D* represent wild-mutant heteroduplexes. (*Bottom*), the nucleotide sequence analyses show nucleotides 127–138 for the wild type and 127–140 for the mutant. The GA repeats are numbered *above* the nucleotide sequence.

on the polyacrylamide gel were visualized by silver staining (Bio-Rad). PCR products were purified by polyacrylamide gel electrophoresis and used for sequence analyses. The sequencing reactions were carried out using the Taq Dye-Deoxy Termination Cycle Sequencing Kit and DNA Sequencer (Model 373) from Applied Biosystems.

The mutation screening showed heteroduplex formation only for exon 2. These heteroduplexes were detectable for every hypocatalasemic (n = 23) but not for the normocatalasemic (n = 26) members of families M, D, and G. No heteroduplex formation was detected in the hypocatalasemic and normocatalasemic members of the other three hypocatalasemic families.

The heteroduplex pattern showed the first band at 268 bp (wild-wild homoduplex), the second at ~270 bp (mutant-mutant homoduplex), and two heteroduplexes at 273 and 304 bp. It is rare to be able to distinguish these four bands so clearly. Separation of the patterns of the four bands in exon 2 were found in the same well when a smaller gel (150 × 150 × 1.5 mm) was prepared with molecular biology-grade polyacrylamide (Bio-Rad) and no sample treatment was used (Fig. 1, middle panel).

The nucleotide sequence analysis showed a GA insertion (Fig. 1, bottom panel) at position 138 of exon 2. This insertion increased the GA repeat number from four to five and caused a frameshift mutation. This frameshift changed the amino acid sequence from position 68 and generated a terminating codon of TGA at position 134. The mutation yielded a truncated protein and the lack of histidine 74, which is required for the binding of a hydrogen peroxide substrate (12). These findings could explain the decreased blood catalase activities of the hypocatalasemic patients (49.2 ± 13.7 MU/L; n = 23) compared with the normocatalasemic family members (107.6 ± 19.5 MU/L; n = 26). The heteroduplex formed from a 268-bp wild-type and a 270-bp mutant PCR product required neither pretreatment nor a special gel for its detection. This simple method was checked when the screening of the 625 normocatalasemic subjects yielded no heteroduplex formation.

In conclusion, a simple heteroduplex analysis of PCR products could be used for screening of GA insertions in exon 2 of the catalase gene. This new syndrome-causing mutation was detected in three of the nine hypocatalasemic families in Hungary. These data confirm the heterogeneity of the acatalasemia/hypocatalasemia detected in Hungary.

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Stability of Several Biochemical Markers of Bone Metabolism,

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The determination of bone metabolism markers is useful in monitoring pharmacological therapy for osteoporosis (1–3). During the course of multicenter clinical studies designed to evaluate the therapeutic efficacy of several drugs, it often is useful to carry out analysis in batches after storing the samples for differing periods, thus reducing analytical variation and cost. This requires knowledge of the behavior of the analyte in the biological matrix in terms of the length and conditions of storage. Unfortunately, these data frequently are lacking, and the information provided by a kit's manufacturer more often than not is contradictory.

Data published relative to long-term storage in biological matrices of bone turnover markers, including the seric NH$_2$-terminal propeptide of type I procollagen (P1NP), the urinary cross-linked N-telopeptides of type I collagen (NTx), and the urinary pyridinium cross-links pyridinoline (PYD) and deoxypyridinoline (DPD), are extremely scarce (4). Therefore, our aim was to systematically study the molecular stability of these analytes in the storage mode most frequently used. In particular, the stability of storage lengths from 1 day to 12 months at temperatures between −80 °C and 23–25 °C were studied.

The biological samples were collected from 10 healthy subjects, 7 females and 3 males, between 25 and 64 years of age (mean ± SD, 36.1 ± 13.2 years), who had fasted from midnight. The venous blood samples were collected between 0800 and 0900, and the serum was separated through centrifugation, divided into 1-mL aliquots in micro test tubes, and stored at 23–25, 2–8, −20, or −80 °C. At the same time, aliquots of the second urine (fasting) of