of bilirubin could be a biological (2) and, as a result, becomes oxidized in vivo by reacting with ROSs. Hence, the oxidative metabolites of bilirubin could be a biological antioxidant and be protective against oxidative stress. To date, the production of biopyrrins may be useful for estimating oxidative stress in vivo, and the measurement of biopyrrins appears to reflect the degree of oxidative stress in vivo; and the measurement of biopyrrins may be useful for estimating oxidative stress. To date, however, the production and excretion of biopyrrins in healthy human subjects have not been critically examined.

The first urine in the morning was collected from 33 healthy volunteers (33 ± 18 years old) and from 14 patients (56 ± 13 years old) who had undergone abdominal operations lasting >5 h. Subjects gave informed consent according to the ethics rules of Kyushu University Hospital. The biopyrrin concentration in urine was corrected by the concentration of creatinine in the same sample. The samples from healthy subjects were collected periodically, 5 to 25 times per person (average, 18), for 1 month. The total number of samples collected was 600. For each individual, the mean (M) of the urine concentration of biopyrrins and its standard deviation (SD) were determined. The SD (defined as SDi/Mi) was used to assess day-to-day variation, i.e., the degree of change of the biopyrrin concentration between days in a given individual. Subsequently, a mean, SD, and CV were calculated for each group of the healthy subjects.

The biopyrrin concentration was determined by subtracting the concentration of the diazo reaction-positive substances (i.e., bilirubin) from the concentration of the anti-bilirubin monoclonal antibody 24G7-reactive substances. The concentration of the anti-bilirubin monoclonal antibody 24G7-reactive substances was measured with a biopyrrin measuring kit (Shino-test), using bilirubin as a calibrator according to the manufacturer’s instructions.

The concentrations (± SD) of biopyrrins in healthy male and female subjects and in the total number of healthy subjects were 0.82 ± 0.29, 0.74 ± 0.11, and 0.79 ± 0.24 μmol per gram of creatinine, respectively. The mean values of the day-to-day CV and SDs of the means in healthy male and female subjects and in the total number of healthy subjects were 0.33 ± 0.08, 0.32 ± 0.06, and 0.33 ± 0.08, respectively. The physiological change of the urine concentration of biopyrrins between days was small, and the concentration seldom fluctuated more than twice the mean value in a given individual.

The concentration of biopyrrins increased markedly from 0.74 ± 0.44 μmol/g creatinine before the operation to 3.37 ± 6.24, 3.66 ± 6.88, and 2.59 ± 2.71 μmol/g creatinine at 1, 3, and 5 days after the operation, respectively. The value decreased to 0.83 ± 0.61 μmol per gram of creatinine at 14 days after the operation. The increase was not simply related to the increase in serum bilirubin because the concentrations of serum bilirubin and urine biopyrrins were not correlated significantly.

We conclude that biopyrrins may prove useful as a practical clinical test for the estimation of oxidative stress in vivo. This work was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Sciences, Sports, and Culture of Japan. We thank Shino-test Corporation for generously providing a biopyrrin measuring kit. We extend special thanks to S. Narayanan (New York Medical College, New York, NY) for critical reading.

References

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Vitamin B₆ Deficiency May Also Be Important

To the Editor:

Takahashi et al. (1) described no changes in bone metabolism markers and a nonsignificant decrease in vitamin K concentrations after low-energy neck or femur fractures in a group of 28 elderly women. Their theory was that vitamin K is required for γ-carboxylation of osteocalcin (2) and that the acute fracture should

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produce changes in its concentration and in bone markers over the 3 days immediately post fracture. There is an extension to this theory that other researchers may find worthy of investigation. Vitamin B₆ is an essential cofactor for the enzyme ornithine decarboxylase, the rate-limiting enzyme in the formation of putrescine, which in turn regulates osteoblast glucose-6-phosphate dehydrogenase activity and thus osteoblast NADPH concentrations (3–6). NADPH is essential for the vitamin K cycle, in which the epoxide form of vitamin K is converted back to the naphthoquinone form, which is required for γ-carboxylation of osteocalcin (2). It is therefore possible that vitamin B₆ status could modulate the effects of vitamin K on bone metabolism.

Although the chain of events described above may seem excessively complicated, there is evidence that vitamin B₆ deficiency in rats reduces bone healing (7) and that vitamin B₆ assayed by HPLC (8) is statistically significantly lower in patients who fracture their hips in low-energy falls than in patients whose hip fractures are elective procedures (9). Further research into the interaction of these two vitamins may be indicated.

**References**


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**Serum Cystatin C, a New Marker of Glomerular Filtration Rate, Is Increased during Malignant Progression**

Cystatin C has recently been shown to be an accurate marker of glomerular filtration rate with advantages over serum creatinine (1, 2). Cystatin C, a potent inhibitor of cysteine proteases, is found mainly in extracellular fluids such as blood, cerebrospinal fluid, and seminal plasma. Its low molecular weight and stable production rate indicate that the blood concentration of cystatin C is determined mainly by glomerular filtration. The production rate of cystatin C is less altered by nonrenal factors than is the production of creatinine, and it has been reported that circulating cystatin C concentrations are not affected by inflammatory conditions or malignancy (3). Our observations, however, have revealed a significant correlation between increased serum cystatin C and malignant progression in melanoma and colorectal cancer.

In malignancy, an imbalance between cysteine proteases and their inhibitors, associated with a metastatic tumor cell phenotype, is thought to facilitate tumor cell invasion and metastasis (4). Numerous studies have provided evidence of substantial increases in mRNA, protein, and the activity of tumor cysteine proteases, accompanied by only moderately increased or unchanged concentrations of intracellular inhibitors (5). Enhanced extracellular secretion of cysteine proteases is another feature associated with tumor cell phenotype. We recently published evidence that high serum concentrations of the cysteine proteases cathepsins B and H are of prognostic importance in predicting the rate of death in colorectal (6) and melanoma cancer (7). These high concentrations

**Fig. 1.** Determination of cystatin C in sera of healthy controls and cancer patients, using ELISA as described by Kos et al. (7).

Ratio 1:2, not significant; ratio 1:3, P = 0.02; ratio 1:4, P <0.0001. Error bars represent mean + 2 SE.