needed to confirm our observation and to clarify its underlying mechanism. Knowledge of this effect may help clinicians to avoid unnecessary and expensive work-ups for suspected hyperparathyroidism.

Grant/Funding Support: None declared.

Financial Disclosures: None declared.

References


Acute Variation of Osteocalcin and Parathyroid Hormone in Athletes after Running a Half-Marathon

To the Editor:

Parathyroid hormone (PTH) and osteocalcin (OC) play important roles in bone remodeling and bone metabolism. Although the physiological functions and clinical significance of these markers are well established, the influence of other biological variables aside from diurnal and seasonal variability has been less well investigated (1). Because little information is available on the kinetics of such markers after physical exercise, we measured PTH and OC in 15 athletes performing a half-marathon run. The study population consisted of 15 healthy trained white males, (mean age, 47 years) who had been engaged in specific endurance training for at least 5 years. Participants performed a 21-km, half-marathon run under competition conditions, while equipped with a heart-rate monitor [mean (SE) VO2 max 85% (3%)]. Prior to the race, preexercise baseline fasting blood samples were collected from the volunteers after a 48-h rest from the last training, 30 min before they warmed up for the race. Post exercise samples were collected immediately after the race, and 3 h, 6 h, and 24 h thereafter. All study participants gave informed consent for being tested, and the study was approved by the ethics
committee. Blood was collected in vacuum tubes containing no additives (Becton Dickinson) and centrifuged at 1500g for 10 min at room temperature, and the serum was immediately analyzed. OC and intact PTH were measured on an Elecsys 2010 by electrochemiluminescence immunoassays (Roche Diagnostics GmbH). We used the N-MID Osteocalcin assay that is specific for intact OC (amino acids 1–49) and the main N-terminal fragment (amino acids 1–43) resulting from proteolytic cleavage after blood collection. Among markers of bone turnover, OC was selected to verify the effects of physical training on osteoblast function, because it may indirectly reflect acute changes in cortisol, and it is currently considered an indirect marker of growth hormone abuse in sports (2). The total assay imprecision (CV) was 1.1%–5.9% for OC and 1.7%–5.5% for intact PTH, respectively (3). The age- and sex-specific reference ranges were 1.6–6.9 pmol/L for PTH and 14–42 µg/L for OC, respectively. Hematocrit and hemoglobin were measured on an Advia 120 (Bayer Diagnostics). Because the estimated decrease in total body water does not differ from the percentage plasma volume change (%PVC), results were adjusted for the %PVC, calculated from pre- and postexercise levels of hematocrit and hemoglobin (4). The Wilcoxon signed-rank test was used to evaluate the significance of exercise-induced variations during the study period. Data with a nongaussian distribution were normalized using a logarithmic transformation before analysis, and the level of statistical significance was set at P < 0.05. Data are presented as geometric mean (SE).

The concentrations of both markers increased significantly by nearly 1.2-fold (OC) and 2.1-fold (PTH) immediately after the run, but 3 h thereafter rapidly returned to values comparable to those measured before the run (Table 1). The number of participants with values above the respective reference ranges remained stable throughout the study period for OC (n = 2, 13%), whereas it decreased from 0 (0%) to 6 (40%) immediately after the run and returned to 0 (0%) 3 h thereafter for PTH.

These results support the hypothesis that a 21-km run produces an acute and transitory increase of both PTH and OC, which is completely reversed 3 h thereafter. Although earlier studies have investigated PTH and OC after strenuous aerobic exercise, especially marathon and ultramarathon running (5), no information is available on the kinetics of these markers following moderately strenuous aerobic physical exercise, such as a 21-km half-marathon run, a distance that is more typically accessible for a general population of active individuals. Although Mouzopoulos et al. observed that PTH concentrations increase by nearly 1.3-fold immediately after 245 km of marathon running, these investigators also reported a significant decrease in OC concentrations. Such differences may be due to different preanalytical conditions (sample matrix and storage) or intensity and duration of the run. The transient suppression in osteoblast function occurring during an ultramarathon run, which has been attributed to increases in cortisol and PTH concentrations (5), might not occur during shorter-distance runs. Perhaps athletes should be advised to run a half or a fraction of a marathon to avoid potential harmful effects of strenuous endurance exercise. Because OC is a marker of growth-hormone abuse in sports (2), such variations should be acknowledged when evaluating the athletes for clinical or antidoping purposes, because these measurement results should be interpreted as physiological responses to exercise rather than pathology or consequences of unfair practices.

Grant/Funding Support: None declared.

Financial Disclosures: None declared.

References

4. Maughan RJ, Whiting PH, Davidson RJ. Estima-

Table 1. Variation of osteocalcin and parathyroid hormone before (pre), immediately after (post), and 3, 6 and 24 h after a 21-km, half-marathon run, in 15 male recreational athletesa

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>3 h</th>
<th>6 h</th>
<th>24 h</th>
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</thead>
<tbody>
<tr>
<td>Plasma volume change, %</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Weight loss, %</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Osteocalcin, µg/L</td>
<td>22.0 (2.5)</td>
<td>27.3 (3.0)</td>
<td>21.6 (2.6)</td>
<td>20.2 (2.9)</td>
<td>22.3 (2.5)</td>
</tr>
<tr>
<td>Parathyroid hormone, pmol/L</td>
<td>3.1 (0.3)</td>
<td>6.4 (0.7)</td>
<td>3.1 (0.3)</td>
<td>3.1 (0.5)</td>
<td>3.2 (0.3)</td>
</tr>
</tbody>
</table>

a Values are presented as geometric mean (SE). Differences from the premarathon values were evaluated by the Wilcoxon signed-rank test.

b P < 0.01, vs the pre-run sample.
Letters to the Editor

Rapid Real-Time PCR Detection of HPdel Directly from Diluted Blood Samples

To the Editor:

An haptoglobinemic patients have been reported to experience severe anaphylactic reactions to transfusions due to the production of anti-haptoglobin (anti-HP) antibodies (1, 2). An haptoglobinemia in patients homozygous for HPdel, which is a deletion of an approximately 28-kb segment of chromosomal 16 extending from the promoter region of the HP (haptoglobin) gene to exon 5 of HPR (haptoglobin-related protein), has been adequately characterized only recently (1). Use of a simple duplex PCR method has detected the HPdel allele in East and Southeast Asian populations at frequencies of 1%-3% but this allele has not been detected in African, West and South Asian, and European populations (1, 3, 4). Thus, diagnosing HPdel homozygosity prior to blood transfusion is the fusion of blood components into individuals from East and Southeast Asian populations is effective for preventing anaphylactoid shock due to anti-HP antibodies. We have developed a simple method that uses a 5' nuclease real-time PCR assay (TaqMan; Applied Biosystems) to detect the HPdel allele without having to isolate genomic DNA. The ethics committee of Kurume University School of Medicine approved this study protocol.

To distinguish alleles, we performed real-time PCR assays that detect the 2 regions that encompass the HPdel breakpoint and the 5' region of HP exon 1, which is deleted in HPdel. The 20-μL PCR reaction contained 200 μmol/L deoxyribonucleoside triphosphates, 1 μL of template (diluted blood or genomic DNA), 0.5 U of Ex TaqHS with its buffer (Takara), and the following primers and TaqMan probes (see Fig. 1 legend for sequences): Hp5'-F and -R primers (450 nmol/L); Hp5'-TaqMan probe (125 nmol/L) for detecting the 5' region of HP; Hpdel-F and -R primers (900 nmol/L); and Hpdel-TaqMan probe (250 nmol/L) for detecting HPdel. The PCR temperature profile was 95°C for 30 s, followed by 50 cycles of denaturing at 95°C for 5 s and annealing and extension at 60°C for 30 s. All oligonucleotides were synthesized by Biosearch Technologies. We monitored amplification progress by monitoring the fluorescence at the end of each cycle with an Mx3000P instrument (Stratagene) with excitation and emission wavelengths of 492 and 516 nm (FAM), and 585 and 610 nm (CAL Fluor Red 610).

With genomic DNA (5 ng/μL) as a template, we used dual-color scatter plots to distinguish individuals previously determined to have the HP/HP, HP/HPdel, and HPdel/HPdel genotypes. Samples with the HP/HP genotype had little FAM fluorescence and plotted along the x-axis, HPdel/HPdel samples had little CAL Fluor Red 610 fluorescence and plotted along the y-axis, and HP/HPdel samples were located between the homozygote samples in the plot (Fig. 1). To the TaqMan real-time PCR mixture, we directly added 1 μL of samples diluted 100-fold with PCR-grade water (previously frozen samples of buffy coat from 47 Indonesians from Surabaya or a freshly drawn blood sample from 1 Japanese individual from Fukuoka). Blood was collected in EDTA-containing tubes (Indonesian and Japanese samples) and in a heparin-containing tube (the Japanese sample). The results from 2 independent experiments showed no discrepancies. In addition, the results obtained with the present TaqMan real-time PCR method were fully concordant with those obtained with a previously described PCR method for the same individuals (i.e., 46 HP/HP individuals and 2 Indonesians with HP/HPdel, Fig. 1) (1). We previously had collected blood samples from 105 Indonesian individuals from Surabaya and had not found the HPdel allele in 58 of these individuals (3); however, in the present study we did find 2 HPdel alleles among the remaining 47 individuals in this population sample. Thus, the HPdel allele is also present in Southeast Asian populat-