lethality was observed in rat pups from administering of phentermine to dams (3), 5 adverse outcomes (stillbirths) occurred in a previous study of 118 pregnant women taking phentermine (4). This case demonstrates the importance of performing confirmatory testing for drugs of abuse and supports a need to include phentermine in confirmatory testing to distinguish its use or abuse from that of amphetamine or other related drugs.

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New Highly Sensitivity Assay Used to Measure Cardiac Troponin T Concentration Changes During a Continuous 216-km Marathon

To the Editor:

We recently measured cardiac troponin T (cTnT) concentrations serially in blood from 10 participants of the Badwater ultramarathon, a continuous 216-km race that takes place under extreme environmental conditions. Details on the Badwater ultramarathon itself, and on the training experience and health condition of the athletes have been reported previously (1). Briefly, the athletes had completed a mean of 43.4 (median 20, range 5–130) marathons and 20.4 (median 15, range 6–80) ultramarathons, and the mean finishing times for the Badwater race were 51.24 h (median 45.2 h, range 43.5–61.4 h). cTnT was measured with a new highly sensitive assay for cardiac troponin T (Hs-TnT) (Roche Diagnostics) on an ELECSYS 2010 automated analyzer that uses chemiluminescence technology. As described previously (2), the interassay CV of this assay is 8% at 10 ng/L and 2.5% at 100 ng/L, and the intraassay CV is 5% at 10 ng/L and 1% at 100 ng/L. The diagnostic range of this assay is 2 to 10000 ng/L.

Blood samples (EDTA plasma) were drawn at baseline, after the first half marathon, after each full marathon distance, and at the finish. In a previous study we tested the third-generation cTnT assay in the same study population and were unable to detect cTnT concentrations above the lower limit of detection in any blood sample before or during the

Nonstandard abbreviations: cTnT, cardiac troponin T; Hs-TnT, highly sensitive assay for cardiac troponin T; BNP, brain natriuretic peptide.
ultramarathon (1). We now report that with the new Hs-TnT assay, cTnT was measurable in all samples including the baseline samples. The time course of individual Hs-TnT values is displayed in Fig. 1A. We observed several patterns of cTnT concentration changes detected with over time with the Hs-TnT assay, including cases with constantly low concentrations without changes as well as cases with an rise of Hs-TnT early after the start of strenuous exercise. In one case, Hs-TnT decreased to a low preexercise concentration while the athlete was still running. The latter case is extremely interesting because this observation suggests a physiological counterregulatory process rather than a simple increase of myocardial damage related to the intensity of exercise. It is tempting to speculate that such an adaptation mechanism could be meaningful in limiting the magnitude of myonecrosis. Previous studies on endurance athletes suggested that training intensity and the type of exercise were major determinants of the rate and magnitude of subsequent troponin release (3, 4). A mechanism that in our opinion may contribute to this adaptation mechanism is release of brain natriuretic peptide (BNP). In animal models, cytoprotective and growth-regulating effects of BNP have been reported (5). BNP has been shown to open adenosine-triphosphate–sensitive potassium channels of myocardial mitochondria via the natriuretic peptide receptor A signaling pathway. Furthermore, BNP exerts counterregulatory and sympathoinhibitory effects inhibiting hypertrophy in cultured cardiac myocytes and angiotensin-II–stimulated collagen synthesis by cardiac fibroblasts. Supporting this hypothesis, in our study population N-terminal pro-BNP, the biologically inactive cleavage product of pro-BNP, increased in 9 of 10 runners (inadequate sampling in 1 case), and peak values of N-terminal pro-BNP were significantly higher (424 vs 126 ng/L, P = 0.0063) in those ultraendurance runners who demonstrated an increase of cardiac troponin above the 99th percentile value as measured with the new more sensitive and precise troponin T assay (Fig. 1B). Although a potential limitation of this study is that blood sampling was not complete in all cases, a consistent change supported by 2 sequential samples was present for the most important observations. Assessment of right and left ventricular performance and hemodynamics would have been informative but was not available in this cohort. Owing to the small number of individuals studied and the heterogeneity of the release patterns, confirmatory follow-up studies are needed. Nevertheless, the observation is interesting and has allowed generation of a hypothesis that should be validated in future studies.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article.
AQP4-IgG Immunoprecipitation Assay Optimization

To the Editor:

The sensitivity and specificity of tissue-based immunofluorescence assays (IFA)\(^1\) is established for detecting neuromyelitis optica (NMO) IgG as an aid to classifying inflammatory demyelinating central nervous system disorders belonging to the NMO spectrum (1): NMO, partial and inaugural forms of NMO [longitudinally extensive transverse myelitis (≥2 vertebral segments radiologically) and recurrent optic neuritis] and pediatric inflammatory autoimmune encephalopathies. Immunoprecipitation assays (IPA) employing recombinant human aquaporin-4 (AQP4), the antigen of NMO, have been described (2, 3). In optimizing the green fluorescent protein (GFP)-tagged AQP4 IPA (2) for high-throughput testing, we encountered cases in which patient IgG bound to GFP and not to AQP4. Here we report our efforts, in a clinical setting, to determine the rate of false positivity and take steps to eliminate it.

From October 1, 2007 to March 31, 2008, the Mayo Clinic Neuroimmunology Laboratory evaluated, by use of GFP-AQP4 IPA, sera from 117 healthy adult control individuals (group 1) and from 5500 patients for whom service NMO-IgG IFA testing was requested. Of these, 557 were IFA-positive (group 2) and 4943 were IFA negative (group 3). We also tested neurologically asymptomatic patients, 58 with hypergammaglobulinemia (group 4) and 27 with, systemic lupus erythematosus or Sjögren syndrome (group 5).

In the course of clinical serological evaluation for NMO-IgG (indirect immunofluorescence assay performed on a service basis in the Mayo Clinic Department of Laboratory Medicine and Pathology), patients were identified. Medical records were reviewed retrospectively. The Institutional Review Board (IRB) of the Mayo Clinic College of Medicine, Rochester, MN, approved the study (IRB 07-007453).

We added 30 μL of serum to duplicate 85-μL aliquots of recombinant human GFP-AQP4 [150 000 GFP counts, solubilized in lysis buffer (700 mmol/L NaCl, 10 mmol/L Tris-HCl pH 8.0, 1 mmol/L EDTA, 0.5% Triton X-100)] from human embryonic kidney—293 cells transfected with pEGFP-AQP4 (plasmid-DNA—encoding enhanced GFP-AQP4) (2) and held 16 h at 4°C. Control specimens contained 4 different pools of normal human serum samples or 1 serum sample

References


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AQP4-IgG Immunoprecipitation Assay Optimization

1 Nonstandard abbreviations: IFA, immunofluorescence assay; NMO, neuromyelitis optica; IPA, immunoprecipitation assay; AQP4, human aquaporin-4; GFP, green fluorescent protein; MuSK, muscle-specific kinase; IRB, Institutional Review Board.