Virtually all samples in this study showed evidence of hemolysis. The median hemoglobin concentration was 35.7 μmol/L [230 mg/dL; range, 8.7–197.6 μmol/L (56–1275 mg/dL)]. Because of the complex nature of the mechanism of hemoglobin interference in bilirubin measurements, there was no direct relationship between hemoglobin concentrations and the magnitude and direction of the interference bias. A plot of the hemoglobin concentration in each sample vs the magnitude of the interference bias, calculated as HPLC-measured bilirubin minus Olympus analyzer- or Leica analyzer-measured bilirubin concentrations, revealed a slope and y-intercept of 0.001 and −7.5 μmol/L, respectively, for the Olympus analyzer procedure and 0.000 and −2.1 μmol/L, respectively, for the Leica bilirubinometer procedure. Neither slope was statistically significantly different from zero ($P > 0.99$).

In conclusion, the measurement of total bilirubin with a Leica direct spectrophotometric procedure provides better agreement with HPLC than does a diazo (lendrassik–Gröf-based) procedure in an Olympus analyzer, and the Leica method provides the advantages of small instrument size, small sample volume, and rapid turnaround. However, it should be noted that none of the specimens in this study was visibly lipemic, and we did not evaluate the effects of lipemia on the Leica and Olympus methods. In addition, all samples were obtained from newborns <4 days of age.

References

Release Characteristics of Cardiac Biomarkers and Ischemia-modified Albumin as Measured by the Albumin Cobalt-binding Test after a Marathon Race, Fred S. Apple, Heidi E. Quist, Angela P. Otto, Wendy E. Mathews, and Mary Ann M. Murakami (Department of Laboratory Medicine and Pathology, Hennepin County Medical Center, University of Minnesota School of Medicine, Minneapolis, MN 55415; * address correspondence to this author at: Clinical Laboratories MC 812, Hennepin County Medical Center, 701 Park Ave., Minneapolis, MN 55415; fax 612-904-4229, e-mail fred.apple@co.hennepin.mn.us)

Numerous studies have monitored the appearance of both cardiac and skeletal muscle proteins and enzymes after short- and long-term exercise regimens. Both animal and human exercise models have attempted to determine whether the stress of long-duration exercise, such as a
marathon race (42.2 km), could cause myocardial necrosis and the release of cardiac-specific proteins, such as cardiac troponin I (cTnI) or T (cTnT) (1–4). In a rat animal model, the severe stress of swimming for 5 h caused microscopic evidence of myocardial necrosis, which led to substantially increased serum cTnT concentrations (1). However, in a less stressful regimen of 1–3 h of swimming, no evidence of ischemic injury and only very minor alterations in serum cTnT concentrations were detected. Middle-aged male marathon runners had increases in inflammatory and prothrombotic markers within 4 h after completion of a race (5). Increases in cTnI were also observed (6). However, no evidence of microinfarction was found by sestamibi imaging, nor was evidence of left ventricular dysfunction found by B-naïtriuretic peptide testing (6).

Ischemia-modified albumin (IMA), measured by the albumin cobalt-binding (ACB) test (7), is reported to predict subsequent cTnI results for patients presenting with symptoms of acute coronary syndromes. Furthermore, studies that included percutaneous transluminal coronary angioplasty as a model of transient myocardial ischemia have demonstrated that serum IMA concentrations, as measured by the ACB test, were increased very early after an ischemic event (8); thus, IMA may be a very early indicator of myocardial ischemia before necrosis. To test this hypothesis in healthy persons at very low risk for cardiac disease but at high risk for skeletal muscle damage, we studied the appearance and clearance characteristics of the IMA marker compared with cTnI, cTnT, and myoglobin in runners after a marathon race.

Nineteen Caucasian runners, 7 men and 12 women, were recruited to participate in this study. Informed consent was obtained after Institutional Review Board approval. The mean age of the runners was 38 years (range, 26–53 years). All participants completed a health questionnaire and described themselves as healthy, without a history of heart disease or diabetes. The mean finish time for the marathon race was 4 h, 49 min, 51 s (range, 3 h, 55 min, 12 s to 6 h, 40 min, 0 s). Blood (heparinized plasma) was obtained before the race (baseline), immediately after the race, within 30 min of finishing, and 24–48 h after the race. The plasma samples were stored under refrigeration and analyzed within 48 h for the IMA marker by the ACB test (5) and for cTnI (Dimension RxL; Dade-Behring), cTnT (Elecsys 2010; Roche), and myoglobin (Elecsys 2010). The upper reference limits for each assay were as follows: ACB, 80 kilounits/L; creatine kinase MB, 5 μg/L; cTnI, 0.1 μg/L; cTnT, 0.03 μg/L; and myoglobin, 110 μg/L. The imprecision (CV) for all assays at these cut points was ≤15%. The results are reported as both medians and means. For each assay, the statistical significance of changes over time was determined by a repeated-measures ANOVA. Comparisons between time points were determined by Fisher protected least significant difference t-tests for multiple comparisons. Statistical significance was defined as P <0.05.

Shown in Fig. 1 are the scatterplots (9) for all of the biomarkers monitored during the study. The median prerace, postrace, and 24- to 48-h concentrations for each marker were as follows: ACB, 74.6, 49.2, and 84.7 kilounits/L; myoglobin, 30, 565, and 52 μg/L; cTnI, 0.0, 0.4, and 0.0 μg/L; and cTnT, 0.01, 0.04, and 0.01 μg/L. All four markers showed statistically significant changes over the time course of the three blood draws (ACB, P <0.0001; cTnI, P = 0.01; cTnT, P <0.0001; myoglobin, P <0.0001). Myoglobin demonstrated a significant increase to 791 μg/L (mean) immediately after the race, but returned toward baseline values by 24–48 h (P <0.01). All runners had increased postrace myoglobin values. Six runners

Fig. 1. Scatterplots for IMA, as measured by the ACB test, and cTnI, cTnT, and myoglobin concentrations before (Pre-Race), immediately after (Post-Race), and 24–48 h (24–48 h) after a marathon race. *, P <0.01 vs prerace; †, P <0.01 vs postrace.
(31.5%) showed increased postrace cTnI concentrations (range, 0.1–0.3 μg/L; P = 0.01). All cTnI values returned to <0.1 μg/L at 24–48 h except the value for one runner, who had a 24- to 48-h value of 0.5 μg/L. Ten runners (52.6%) showed an increase in postrace cTnT concentrations (0.03–0.1 μg/L; P < 0.01). All but one runner (at 0.04 μg/L) had 24- to 48-h postrace cTnT values that returned to baseline values of <0.01 μg/L. For the ACB test, at baseline six runners (31.5%) demonstrated minor increases (81–91 kilounits/L). Immediately after the race, all values were within reference limits. However, at 24–48 h after the race, all runners demonstrated a significant increase (P < 0.01) in ACB values, from a mean of 49 kilounits/L immediately after the race to 84 kilounits/L at 24–48 h, with 12 runners (63.1%) having values above the upper limit of the reference interval (range, 81–110 kilounits/L). The ACB test results 24–48 h postrace were significantly higher than both baseline and immediate postrace values (P < 0.01). We found a poor correlation (r = 0.23) between changes in albumin and ACB concentrations across pre- and postrace time points.

We hypothesize that the increase in the ACB test values 24–48 h postrace were not of myocardial origin, but could be attributed to either gastrointestinal ischemia (9) or skeletal muscle ischemia (2), which occur during long-distance running. Gastrointestinal blood loss, which occurs 12–72 h after marathon racing owing to an ischemic mechanism, is a common finding in distance runners after a marathon race (10). The minor baseline ACB increases observed in the six runners may have been a result of the last, strenuous training run that each runner performed 2–3 days before baseline blood sampling. As expected, the physical trauma of running was responsible for the large increases in myoglobin release from skeletal muscle. However, the immediate increase in myoglobin was not associated with an immediate IMA increase. Therefore, we do not think that the acutely induced skeletal muscle injury was mechanistically responsible for the increased ACB test results observed at 24–48 h.

Only a poor correlation was observed between increases in cTnI or cTnT and changes in ACB test values. Although an increase in cardiac troponin reflects myocardial cell necrosis (irreversible damage), it is not clear what mechanism was responsible for the minor increases in cTnI and cTnT observed in the runners. Our findings show concordant increases for both cTnI and cTnT and agree with values observed in ultraendurance athletes [both cTnI and cTnT were increased in 6 of 23 athletes immediately after the Ironman Triathlon (11)] and with results from other postrace triathlon studies that included testing for cardiac troponin concentrations (2–4, 6). As in our study, cardiac troponin values normalized within 24–48 h. We did not study the cellular mechanism response for the release of troponin, although it is likely different from that produced after a myocardial infarct. A possible role of catecholamine-induced myocardial injury after exercise has been proposed (12). Although numerous studies have demonstrated the important prognostic role that increases in serum cTnI and cTnT concentrations play in predicting short- and long-term cardiac events in patients presenting with ischemic symptoms (13, 14), no data are available to estimate whether a transient increase in troponin after a stressful bout of exercise in a healthy athlete alters the risk of future cardiac events. Although the overall risk of death from coronary heart disease is decreased twofold in physically active people (15), heavy physical exercise is recognized as a factor that can trigger severe myocardial events (16).

The major proposed role of the ACB test is in the early detection of myocardial injury in patients with coronary symptoms (7). Cardiac troponin testing, either cTnI or cTnT, has been recommended by both the cardiology (17, 18) and the laboratory medicine (19) communities as the new standard for detection of myocardial injury. In the setting of ischemia, patients who demonstrate an increased serum cardiac troponin concentration are classified as having sustained a myocardial infarction, whereas those patients with a normal troponin value are classified as having unstable angina (18, 20). In one multicenter study, admission ACB test results for acute coronary syndrome patients demonstrated a high, negative predicative value and high sensitivity for predicting troponin-negative or -positive findings 6–24 h after presentation (7). In the present study, the stress of running a marathon race did not increase IMA in the short term, suggesting that the test may be used in such patients when myocardial injury is suspected. Moreover, the data suggest that myocardial ischemia was not present in these runners. In contrast, acute coronary syndrome patients display a continuum of sequential pathologic processes involving the disruption of vulnerable atherosclerotic plaque, which ultimately leads to myocardial cell death. The mechanism of release of cardiac troponin, thought to be caused by irreversible cell death, appears to differ from the mechanism that increases ACB test values, which has been proposed as a marker of myocardial ischemia. A better understanding of the pathophysiology underlying the myocardial injury that leads to a transient increase in cardiac troponin values in exercise-stressed runners is needed. The significant postrace IMA increase at 24–48 h, however, does suggest some form of ischemia.

Our current findings, although preliminary, show that the IMA marker is likely not increased because of heart or acute skeletal muscle ischemia produced by significant exercise, at least in the period immediately after exercise. IMA increases 24–48 h postrace may be attributable to gastrointestinal ischemia or perhaps a delayed response to skeletal muscle ischemia. Additional studies are needed to more clearly delineate the specificity and sensitivity of the ACB test in a broader group of ischemic patients without myocardial involvement. In the context of a marker that might be useful for investigating patients with possible acute coronary syndromes, it is important to recognize that the IMA marker, unlike myoglobin, does not increase in the short term as a result of acute skeletal muscle ischemia or trauma.
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References


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Moderately increased plasma total homocysteine (tHcy) concentrations have been associated with an increased risk of atherothrombotic vascular events (1). Disturbances in homocysteine metabolism have also been reported as a possible risk factor for early pregnancy loss and congenital birth defects, such as neural tube defects, as well as for maternal obstetric complications (2).

Reference intervals for healthy maternal and newborn populations are scarce; in particular, we could not find data on large samples of women at delivery. Rajmakers et al. (3) reported tHcy results for samples collected at delivery or 4 h before cesarean section on 35 women. Böhle et al. (4) and Malinow et al. (5) measured tHcy at delivery in 60 and 35 women, respectively, and Bjerke Monsen et al. (6) reported results for 169 samples collected between 96 to 108 h after birth. Available data on newborns include the results from one large study in Italy (7) and a few smaller studies (3–6), among which only one is from North America; it included 35 women and their newborns (5). Mean tHcy values were quite different among these studies.

Genetic, nutritional, and lifestyle factors are believed to influence tHcy concentrations (8, 9). Among the genetic factors, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C gene polymorphisms are potentially important. Supplementation of the diet with folate as well as smoking and caffeine consumption are among other factors that can affect tHcy concentrations. None of the studies cited above took the effect of these factors into account.

The goals of our study are (a) to provide reference values for tHcy measured within 48 h of delivery from a large unselected sample of women who gave birth to babies born at or above the 10th percentile for gestational age and sex; (b) to provide similar values for their newborns; and (c) to study the impact on maternal as well as newborn tHcy concentrations of common MTHFR genetic polymorphisms as well as nutritional factors.

We performed a hospital-based case-control study of intrauterine growth restriction in which all live-born singleton cases seen over a 2-year period (mid-1998 and mid-2000) were matched for sex, gestational age, and race to a live newborn control whose weight was at or above the 10th percentile based on gestational age and sex, as determined by Canadian population standards (10). Cases and controls were born at the same hospital and...