[P\textsubscript{i}] and [PCr\textsubscript{j}] is (other things being equal) a decrease in total cell phosphorus of ~3.5 mmol/L. Because the volume of muscle intracellular fluid is at least twice that of extracellular fluid, this represents (very approximately) 8 mmol of phosphorus per liter of extracellular fluid. In the short term, at least, this decrease in cell [P\textsubscript{i}] might represent a mechanism for partially “buffering” plasma [P\textsubscript{i}] against P\textsubscript{i} depletion. Such a mechanism makes no adaptive sense, however, if the consequent fall in cellular [P\textsubscript{i}] is deleterious. Stimulation and recovery studies suggest that there is no major impairment of mitochondrial and glycolgenolytic function. As in rats injected with PTH (11) and in uremic rats (9), the substantial decrease in cell [P\textsubscript{i}] at rest is not associated with a significant functional bioenergetic abnormality in vivo. Thus muscle can tolerate a reduction in resting cell [P\textsubscript{i}] of as much as 30–40% without exhibiting bioenergetic abnormalities. Although plasma [P\textsubscript{i}] bears no simple relationship to intracellular [P\textsubscript{i}], the significant bioenergetic defects sometimes reported in hypophosphatemia (6, 7) are unlikely to be a simple consequence of reduced cell [P\textsubscript{i}], unless resting cell [P\textsubscript{i}] decreases by >40% below control values.

In summary, mild dietary phosphorus depletion reduces muscle cell [P\textsubscript{i}] in the absence of significant hypophosphatemia. There is also a decrease in cell [PCr\textsubscript{j}], which tends to maintain resting phosphorylation potential and therefore also the free energy of ATP hydrolysis. These changes, which may have the temporary effect of partially buffering plasma [P\textsubscript{i}], are not associated with significant impairment of muscle bioenergetics. These results provide further evidence that, as shown before (3), plasma [P\textsubscript{i}] measurements in isolation may tell us very little about intracellular [P\textsubscript{i}] in skeletal muscle.

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References

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Immunoselection vs Immunofixation for Light Chain Typing

To the Editor:

I was interested to read the Letter of Su et al. (1) regarding the failure of anti-\lambda antibodies to precipitate a monoclonal IGA \lambda in immunofixation electrophoresis. The inability of some monoclonal IGA to react in gel precipitation assays with anti-\lambda antibodies has long been known (2) and, in fact, is not an uncommon finding in clinical laboratories. The “unreactive” light chains are probably hidden in the immunoglobulin molecule.

In our hands, immunoselection, which involves a coprecipitation of the monoclonal protein together with polyclonal immunoglobulins, can identify light chains when other techniques cannot. The “pragmatic approach” of using different antisera when such situations occur (1) is not always successful. How many different antisera should one try in immunofixation before concluding that only heavy chains and no light chains are present? I have noted some monoclonal IGA that could be not typed (i.e., their light chain was undetermined) using the same antibodies in both assays (3). Therefore, I believe that immunoselection is the reference technique for determining the light chain (type) of a monoclonal immunoglobulin (especially IGA) and hence for ruling out heavy-chain diseases (4). Once, immunoselection is generally more difficult to perform than immunofixation because ready-made commercially products are available for only the latter assay. However, it is not difficult to make immunoselection plates and perhaps these also will become commercially available.

References

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The authors of the letter referred to reply:

To the Editor:

We want to emphasize that alpha heavy-chain disease is very uncommon in the US and is marked by distinctive clinical features. Accordingly, any immunofixation procedure that results in an interpretation of "heavy chain disease" should be carefully scrutinized. A review of serum protein immunofixation electrophoresis procedures performed at the University of Michigan Medical Center since 1989 revealed 247 cases in which an IgA monoclonal gammopathy was identified. In 3 cases two different anti-lambda reagents were required to identify the light chain component. We have encountered no failures of anti-kappa light chain reagent and no cases of alpha-chain disease. We agree that the immunoselection technique may be useful, and in fact necessary, to identify a light chain component, but such instances are uncommon.

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Validation of Abbreviated Oral Methionine-Loading Test

To the Editor:

Hyperhomocysteinemia 4 to 6 h after an oral methionine load may identify individuals at increased risk for atherothrombotic sequelae despite normal fasting total plasma homocysteine concentrations (1, 2). An excess prevalence of postmethionine-load hyperhomocysteinemia, in the absence of fasting hyperhomocysteinemia, has also been described in women with a history of giving birth to children with neural tube defects (3). Administration of the methionine-loading test in both epidemiologic and clinical settings is limited by the prolonged duration of existing protocols. In an effort to facilitate expanded application of the methionine-loading test, we attempted to validate a shortened (i.e., 2-h) protocol.

Three groups of adult volunteers were studied at The Framingham Study, or at the General Clinical Research Center at Tufts New England Medical Center (NEMC). The participants included 40 Framingham Study or NEMC employees (26 women, 14 men; mean age 43 years, age range 22-75); 6 obligate heterozygotes (3 women, 3 men; mean age 44 years, age range 29-53) for deficiency of cystathionine synthase (CS; EC 4.2.1.22) who were free of clinical or laboratory evidence of cardiovascular, renal, and hepatic disease; and 21 rheumatoid arthritis patients (14 women, 7 men; mean age 53 years, age range 35-76). Informed consent was obtained from all study participants.

Phlebotomy was performed after an overnight fast (10 to 14 h), and at 2 and 4 h after receiving an oral load (0.1 g per kg of body weight) of \( \text{L-methionine} \) (Ajinomoto, Teaneck, NJ) mixed in 200 mL of fruit juice. After the fasting phlebotomy and oral methionine load, those evaluated at the Framingham Study (i.e., 23 of 40 of the employee volunteers, and all 6 of the CS deficiency heterozygotes) received a standardized snack low in methionine (<15 mg); the remaining 17 employee volunteers and the 21 rheumatoid arthritis patients evaluated at NEMC were given only water. All whole-blood specimens for homocysteine analyses were drawn into evacuated blood-collection tubes containing EDTA, and without delay cooled to 4°C. Within 2 h of collection, the whole blood was centrifuged in a refrigerated centrifuge, and the separated EDTA plasma was aliquoted and cryopreserved at \(-70^\circ\text{C}\) for <2 months until analysis.

Total plasma homocysteine was determined by HPLC with fluorescence detection in a modification of the method originally described by Araki and Sako (4). Inter- and intraassay CVs for this assay are routinely \(<7\%\) and \(<5\%\), respectively.

Linear (Pearson \( r \)) and rank order (Spearman \( \rho \)) correlations between 2-h and 4-h plasma total homocysteine concentrations were respectively 0.97 and 0.92 for the rheumatoid arthritis patients (n = 21), 0.94 and 0.95 for the Framingham and USDA-HNRC employee volunteers (n = 40), each 0.99 for the CS deficiency heterozygotes (n = 6), and 0.96 and 0.94 for the pooled groups (n = 67). As shown in Fig. 1, the 2-h plasma homocysteine concentration accounted for \( >92\% \) of the variability in 4-h plasma homocysteine concentration. In addition, the correlation between the 2-h value minus the fasting value and the 4-h value minus the fasting "delta" total plasma homocysteine concentrations for the pooled groups was \( r = 0.95 \). Four individuals with a normal fasting total plasma homocysteine (<16 \( \mu \text{mol/L} \); see ref. 5) had a 4-h postmethionine-load total plasma homocysteine exceeding the 90th percentile distribution for the pooled groups, i.e., >50.6 \( \mu \text{mol/L} \), a value consistent with increased risk for atherothrombotic sequelae (1). These same four individuals also had 2-h total plasma homocysteine concentrations exceeding the 90th percentile (i.e., >37.7 \( \mu \text{mol/L} \)), indicating 100% concordance in these four subjects for hyperhomocysteinemia at 2 and 4 h postmethionine loading.

In view of our combined experience with \(-300\) 4-h methionine-loading tests (A. Bostom, unpublished observations), the 2-h loading test offers...