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Serum Potassium Is Unreliable as an Estimate of in Vivo Plasma Potassium, *Andrew J. Hartland* and Richard H. Neary* (Department of Clinical Biochemistry, Central Pathology Laboratory, North Staffordshire Hospital NHS Trust, Hartshill Rd., Stoke-on-Trent ST4 7PA, UK; * author for correspondence: fax 44 1782 554646)

The in vitro release of potassium from cells and platelets during blood clotting [particularly in patients with blood dyscrasias *(1, 2)*] increases serum potassium, on average, by 0.4 mmol/L *(3)*. This difference is considered to be independent of collection tube type *(4)*. Following a change in the supplier of collection tubes, however, we observed an increase in the frequency of pseudohyperkalemia, which prompted a formal evaluation of the serum-plasma difference and the effect of different tube types.

Free-flowing blood, taken without a tourniquet to avoid venous stasis, was collected from 40 hospital outpatients. Paired samples of clotted blood (5 mL) were collected in random order into the types of tubes listed in Table 1. One tube of each pair was selected at random, to prevent systemic bias, and centrifuged, and the potassium concentration was determined without delay (time 0). Potassium was measured using an automated discrete analyzer with an ion-selective electrode (Bayer Diagnostics). The second tube of each pair was left to stand for 3 h at room temperature before centrifugation and analysis (time+3), representing a typical delay in sample transport to the laboratory. The analytical imprecision (CV) was <0.5% for both serum and plasma within and between batches (n = 30). The significance of the differences between serum and plasma was tested using the Student *t*-test, and the relationship between this difference and the plasma potassium concentration was tested using least-squares linear regression.

The mean plasma potassium concentration was 4.16 mmol/L (SD, 0.33 mmol/L; range, 3.49–4.77 mmol/L). When the blood stood for 3 h before centrifugation (time+3), the mean plasma concentration increased by a nonsignificant 0.01 mmol/L (95% confidence interval, −0.088 to 0.108 mmol/L). The differences between serum and plasma at baseline and at (time+3) are shown in Table 1. The mean difference between the serum and plasma potassium concentrations at baseline was similar for the different tube types (0.324–0.379 mmol/L). However, the variation between patients in the amount of potassium released during clotting was marked, as shown by the wide confidence intervals of the plasma-serum difference, which spanned 1.08 mmol/L for one type of sample tube (tube E). The 3-h delay in separation of serum from clot increased this difference, with the 95% confidence intervals spanning up to 1.5 mmol/L (tube C), although in one type of tube, the mean increased by a nonsignificant 0.079 mmol/L (tube D). The serum-plasma and the baseline to (time+3) differences were not related

Table 1. Effect of specimen tube and delay in separation on potassium concentration.

Tube	Type	Mean difference (95% confidence interval) in potassium concentration from plasma at time 0, mmol/L		Significance of increase from baseline to 3-h sample, *P*
		Time, 0 h	Time, + 3 h	
A	Plasma (Greiner)		0.010 (−0.09 to 0.11)	NS[a]
B	Glass (Labco)	0.324 (0.00–0.64)	0.469 (0.04–0.90)	0.0001
C	Plastic + clotting accelerator (Greiner)	0.370 (−0.04 to 0.78)	0.469 (−0.32 to 1.28)	0.0002
D	Plastic + clotting accelerator + gel (Becton Dickinson)	0.345 (0.02–0.67)	0.406 (0.15–0.67)	NS
E	Plastic + clotting accelerator + gel (Greiner)	0.326 (−0.22 to 0.87)	0.405 (−0.01 to 0.87)	<0.0001

[a] NS, not significant.

to the plasma potassium concentration or blood platelet count.

The difference between serum and plasma potassium concentrations is well known, but we believe the clinical significance of the intra- and interindividual variability of the difference is not fully appreciated. This variability differs between types of blood collection tubes, possibly because of the extent of clot retraction induced by the surface of the tube or the clotting accelerator. Even for the sample tube showing the least variable potassium release (tube D; Becton Dickinson), serum remains an unsuitable medium for managing patients in whom potassium homeostasis is important, particularly because most standard medical texts base management on plasma concentrations *(5–7)*. The extent of potassium release during clotting or during delays in the separation of serum from cells means that a true plasma potassium in the middle of the reference interval (4.16 mmol/L) may range from 3.82 to 5.44 mmol/L in a simultaneously taken serum sample (tube C); the variation is unpredictable, unrelated to the plasma concentration, and additive to the inherent biological variation in potassium concentrations. A reference interval derived from serum samples would be wider than the interval derived from plasma and would cause truly abnormal concentrations to be masked, e.g., hyperkalemia associated with a small release on clotting or hypokalemia associated with a large release on clotting.

These studies were conducted on hospital outpatients, but because we instituted a policy of requesting plasma when a serum result fell outside the reference interval, we have observed even greater differences in hospital inpatients with disorders, such as diabetic ketoacidosis, in which the potassium balance may be critical.

Plasma is the preferred medium for the determination of potassium concentration, although it is not ideal for many other biochemical analyses. When serum is to be used, laboratories should be aware of the additional variability observed with this medium and the extent to which this is affected by their choice of sample tube.

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Genotyping Method for Point Mutation Detection in the Intestinal Fatty Acid Binding Protein, Using Fluorescent Probes, *Jennifer R. Galluzzi* and Jose M. Ordovas* (Lipid Metabolism Laboratory, USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington St., Boston MA 02111; * author for correspondence: fax 617-556-3103, e-mail RAMOS_LI@hnrc.tufts.edu)

The intestinal fatty acid-binding protein (IFABP) is located in the intestine and is involved with long-chain fatty acid transport and metabolism *(1)*. The *FABP2* gene at chromosome 4q28-31 encodes IFABP. Genetic variation at this locus could lead to altered fatty acid absorption and energy metabolism. A common point mutation (G→A) in the gene for IFABP, with an allele frequency of the A allele of ~0.29 *(2)*, generates an amino acid substitution at codon 54 (alanine→threonine), which was found to be associated with insulin resistance and increased fat oxidation in Pima Indians *(2)*. Recently, genetic variation at this locus has been reviewed in terms of plasma lipid response to diet *(3)*. The current evidence indicates that the IFABP threonine variant is often associated with a more deleterious phenotypic expression, i.e., impaired glucose tolerance, obesity, and altered lipid and lipoprotein profiles *(3)*. In vivo studies also support a functional difference between the alanine- and threonine-containing proteins: Caco-2 cells that express the threonine-containing protein transport long-chain fatty acids and secrete triglycerides to a greater extent than cells that express the alanine-containing protein *(4)*. Therefore this variant is an obvious candidate for examining this gene by the use of diet interactions.

The established procedure for genotyping the G→A mutation includes restriction digestion with *Hha*I, which cleaves the natural restriction site in the wild-type (G54) PCR product *(2)*. The restriction enzyme digestion is then followed by electrophoresis on a 3.5% agarose gel. We report here the successful implementation of an alternative method for genotyping this point mutation, using the Perkin-Elmer/Applied Biosystems 7700 Sequence Detection Systems (SDS) and TaqMan reagents.

The 7700 SDS is a combination of a thermal cycler and a laser-induced fluorescent detector. The technique also involves the use of probes, which are labeled at the 5′ end with a reporter fluorescent dye and at the 3′ end with a fluorescence quencher *(5)*. Two probes are used; one probe is complementary to the wild-type DNA strand, and the other probe is complementary to the DNA strand with the G→A mutation. The two probes have different reporter dyes, 6-carboxy-fluorescein (FAM) and VIC®, which are attached to their 5′ ends. The dye 6-carboxytetramethylrhodamine (TAMRA) is used as the quencher dye and is attached to the 3′ end of each probe. TAMRA will suppress fluorescence from the reporter when the probe is intact *(6)*. During the PCR reaction, the probe will hybridize to its complimentary target sequence in the PCR product. The AmpliTaq Gold DNA polymerase will cleave with its 5′-3′ nuclease activity the TaqMan probe that is hybridized *(6)*. This cleavage will release the