Overestimation of Plasma Nonesterified Fatty Acid Concentrations in Heparinized Blood  

To the Editor:

With much interest have we read the important study on the use of glucose-insulin-potassium (GIK) in diabetic patients undergoing coronary artery bypass grafting by Lazar and coworkers. A potential mechanism underlying the beneficial effect of GIK administration includes metabolic modulation of postischemic myocardium. Lowering of the plasma nonesterified fatty acid (FA) concentration by GIK, associated with mitigation of the noxious effect of high levels of circulating FA on the heart, might be one of the mechanisms responsible for the favorable outcome of GIK treatment. This was an obvious reason for Lazar et al to analyze plasma FA levels throughout their study. Considering the recently renewed interest in GIK for the prevention or treatment of posts ischemic dysfunction, one might expect more articles in the future that report plasma FA levels.

A pitfall in the accurate measurement of FA levels in blood samples, still frequently encountered, is the ongoing ex vivo lipolysis in the test tube, especially after the in vivo administration of heparin. Heparin releases endothelial lipoprotein lipase (LPL), thereby promoting lipolysis of plasma triacylglycerols to FA in vivo. Without special precautions, lipolysis continues ex vivo, which may lead to substantial overestimation of plasma FA levels. To prevent this phenomenon, direct inhibition of ongoing lipolysis in the test tube is required immediately after blood sampling. Because cooling or freezing of plasma samples did not appear to adequately prevent ex vivo lipolysis, several pharmacological techniques have been developed to obtain inhibition of lipolysis of triacylglycerols. First to have been investigated was the use of diethyl p-nitrophenyl phosphate, (Paraoxon, Roche), a cholinesterase inhibitor neurotoxin that only partially blocks plasma lipolytic activity but has potent neurotoxic properties that make it difficult to handle. Another technique developed in the 1990s makes use of a potent LPL inhibitor, tetrahydrolipstatin (Orlistat, Sigma-Aldrich), that is both safe and easy to handle. In 2000, Krebs et al reported a significant reduction of plasma FA levels of >28% caused by the addition of tetrahydrolipstatin to heparinized blood samples.

Lazar et al reported hazardously high peak FA concentrations (mean values in the order of 1.3 mEq/L in both the GIK and control groups). These samples were taken before extracorporeal circulation, ie, after heparinization. Because the authors did not describe how their FA measurements were done, the possibility cannot be excluded that the FA levels described in their article were affected by an artifact, resulting from a laboratory technique in which plasma FA levels were assessed in the absence of an LPL inhibitor. This would make it difficult to draw conclusions about the pathophysiological impact of their findings. However, although the peak FA concentrations reported by Lazar et al might be based on an overestimation, the beneficial effect of GIK on perioperative outcomes in their study population of diabetic coronary artery bypass graft patients is definitely not.

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**Response**

We greatly appreciate the comments from Drs Visser, van Wezel, and colleagues. We were, of course, particularly interested in the effects of GIK on free fatty acids (FFA) as well as glucose metabolism. At each time point, samples were chilled and subsequently separated and frozen. We did not utilize inhibitors of lipoprotein lipase (LPL) and agree that future studies might benefit from use of these agents. We continue to believe that even with this potential improvement, the trends over time and relationships between control and GIK subjects would persist. The first observed trend was the rise in FFA after baseline before cardiopulmonary bypass (“pre CPB” in Figure 31). We agree that this rise was almost certainly due to the heparin (3 mg/kg)–induced stimulation of LPL. The second trend was the subsequent decrease in both control and GIK subjects at time “0” in the intensive care unit; this observation is consistent with the short-lived effect of heparin on 1 of the 6 time points measured. The third trend was the subsequent relative decrease in FFA in patients receiving GIK compared with controls (time 0 and 6 hours in Figure 31). This decrease could have been due to either insulin-induced inhibition of endogenous lipolysis or cellular transport of FFA. Because the heparin effect seems to have abated, we don’t believe that LPL inhibitors would have substantively changed this observation. We believe each of these 3 trends would persist, even if ex vivo lipolysis were minimized by inhibitors. The major benefit of inhibiting LPL would be to determine a threshold level of toxicity in vivo FA to myocardium. This may well be a fruitful area of future research. In our study, we have tried to focus on the trends and comparisons without emphasizing the absolute concentrations. In our future studies, we plan to incorporate inhibitors of LPL in our assays as we attempt to correlate changes in FFA with markers of inflammation and oxidative stress to better define the mechanisms for GIK’s favorable effects.
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