clearance. Although there were no differences between the groups for the absolute values at these time points, the relative changes from baseline are of greater clinical interest, and for urinary neutrophil gelatinase-associated lipocalin (the primary outcome measure), the groups moved in opposite directions.

Competing Interests

The author has a relationship with or consults for the following companies and organizations that have an interest in intravenous fluid therapy: U.S. Food and Drug Administration (Bethesda, Maryland); U.S. National Heart, Lung, and Blood Institute/National Institutes of Health (Bethesda, Maryland); U.S. Department of Defense (Frederick, Maryland); TerumoBCT (Lakewood, Colorado); HbO_2 Therapeutics (Souderton, Pennsylvania); and Octapharma USA (Hoboken, New Jersey). The author helped design a multicenter clinical trial (Gandhi SD, Weiskopf RB, Jungheinrich C, Koorn R, Miller D, Shangraw RE, Prough DS, Baus D, Bepperling F, Warltier DC: Volume replacement therapy during major orthopedic surgery using Voluven (hydroxyethyl starch 130/0.4) or a hetastarch. Anesthesiology 2007; 106:1120–7) sponsored by Fresenius-Kabi (Bad Homburg, Germany), participated in a review of tetrastarches (Van Der Linden P, James M, Mythen M, Weiskopf RB: Safety of modern starches used during surgery. Anesth Analg 2013; 116:35–48), and has received reimbursements for travel expenses and honoraria from Fresenius-Kabi for presentations. In the past, the author has consulted for the following companies that had an interest in development of hemoglobin-based oxygen carriers: Somatogen (Boulder, Colorado), Hemosol (Mississauga, Ontario, Canada), Sangart (San Diego, California), and OPK Biotech (Cambridge, Massachusetts). The author was project/corporate Vice-President, Chief Medical Officer Biopharmaceuticals, and Executive Scientific Advisor at Novo Nordisk A/S (Bagsvaerd, Denmark) 2005–2007. No one from any of these organizations had knowledge of, influenced, or participated in the writing of this letter.

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In Reply:

We are grateful to Drs. Priebe, Xue, and Weiskopf for their interest regarding our manuscript entitled Lack of Nephrotoxicity by 6% Hydroxyethyl Starch 130/0.4 during Hip Arthroplasty: A Randomized Controlled Trial,[1] which appeared in the November 2014 issue of Anesthesiology. Further, we thank for their complimentary words and remarks. We will answer the queries starting with Dr. Priebe, then Dr. Xue and finally Dr. Weiskopf.

Dear Dr. Priebe, you request additional information regarding urine 4 in terms of plasma creatinine and creatinine clearance. However, urine 4 was obtained just before discharge and was a "spot urine," *i.e.*, not a urine collection over time. Thus, creatinine clearance could not be calculated. Additional analyses of urine and blood samples could have contributed with further information in the postoperative period and during follow-up, but this was not included in the protocol.

The study design allowed us to compare the effect 6% hydroxyethyl starch (HES) 130/0.4 and isotonic saline 0.9% on urinary neutrophil gelatinase-associated lipocalin (u-NGAL) during hip arthroplasty. We can conclude that no difference existed between the two solutions, but an increase was seen in u-NGAL in both infusion groups in urine 4. We used isotonic saline 0.9% as control fluid, because it had the same chloride content of 154 mmol similar to the intervention fluid. We agree that a possible nephrotoxic effect of the chloride component in isotonic saline 0.9% is interesting, and further studies are necessary to clarify this aspect. However, other studies that were comparing a balanced solution, *i.e.*, lactated Ringer's solution or similar to a chloride-rich solution, found no differences in u-NGAL in the groups.[2,3]

Dear Dr. Xue, we used a cutoff value of 100 ng/ml for u-NGAL. We wanted to see whether 6% HES 130/0.4 inflicted none, mild, or severe renal injury compared with isotonic saline 0.9%. There were nine *versus* seven patients in the HES *versus* saline group with a u-NGAL value more than 100 ng/ml at discharge. Thus, no difference existed between the groups.

The study was not designed to compare the occurrence of acute kidney injury (AKI) between HES 6% 130/0.4 and isotonic saline 0.9%. It goes without saying that a huge number of subjects had to be included, if AKI should be the primary effect variable. We agree that fulminant AKI is a seldom event after noncardiac surgery, but the outcome in our study was differences in renal markers specific for renal injury, *i.e.*, u-NGAL, plasma creatinine, urine output, and creatinine clearance. So, our study was powered to find a difference in these markers and not to find a difference in the incidence of AKI. When evaluating HES-induced renal failure, it is important to differentiate between a surgical population and a septic one. The findings in severe sepsis are not applicable to surgical patients.[4] Further, there are numerous pharmacokinetic differences between the generations of starches and the findings of side effects. The elder generations of starches cannot be compared with the latest generations of starch.[5] Until now, no evidence exists of a perioperative renal impairment after tetrastarch infusion in subjects with normal renal function before surgery.[6–8]

In a previous study, the follow-up was 28 days after HES infusion, and no signs were detected of HES-induced renal impairment.[9] We are convinced that we would have seen signs suggestive of renal injury within the 14 days of follow-up in the present study, if there had been any.

Dear Dr. Weiskopf, thank you for the complementary words. Due to space limitations, we did not publish the absolute or relative changes from baseline to the follow-up.

Among the absolute changes between baseline and follow-up, we found only a significant difference between the groups for creatinine clearance ($P < 0.01$), whereas u-NGAL, u-$NGAL_{CR}$ (urinary neutrophil gelatinase-associated lipocalin adjusted for creatinine), and urine output did not deviate significantly. Among the relative changes, calculated as (follow-up – baseline)/follow-up × 100, only creatinine clearance deviated significantly ($P = 0.02$), whereas no significant differences were found in u-NGAL, u-$NGAL_{CR}$, and urine output. Thus, creatinine clearance increased slightly in the HES group and was unchanged in the saline group.

Competing Interests

The authors declare no competing interests.

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Being Conscious of Methodological Pitfalls in Functional Brain Network Analysis

To the Editor:

We have read with interest the work by Khodayari-Rostamabad *et al.*,[1] but we would like to point out that volume conduction and uncorrected network measures may have influenced the findings described in this study.

In this double-blinded, placebo-controlled, crossover study, Khodayari-Rostamabad *et al.* performed coherence and graph theoretical ("small world") analyses of electroencephalogram recordings to characterize the effects of remifentanil on the functional brain network in healthy subjects. A reduction of mean coherence was found in the α (8 to 12 Hz) and β1 (12.5 to 18 Hz) frequency bands after infusion of remifentanil, whereas no differences were found after placebo infusion. Network analysis revealed an increase in path length (*i.e.*, decreased integration) and decrease in clustering coefficient (*i.e.*, decreased segregation) in the same frequency bands. A negative correlation was found between the path length in the α frequency band and the continuous reaction time index, which was used as a measure of sustained attention and vigilance. On the basis of these findings, they stated that infusion of remifentanil disrupts the complex cortical network, which was associated with reduced attention.

The field of functional connectivity studies is growing exponentially and has increased our understanding of cognition, neuropsychiatric diseases, and physiological effects of pharmacological agents. Simultaneously, methodological limitations of initial approaches of functional connectivity and network analysis of electroencephalogram recordings have been described, which may potentially bias results.[2] Some concerns may apply to the study by Khodayari-Rostamabad *et al.*

First, coherence was used to calculate functional connectivity, which is likely to be affected by volume conduction, and therefore, coherence might be influenced by alterations in the power spectrum. Multiple electroencephalogram channels will pick up activity of a single source because of transmission of the signal through tissue between the cortex and the electrodes (*i.e.*, volume conduction) and spreading of the electrical field. As a consequence, coherence and correlation can give erroneous estimates of functional connectivity.[3] Spectral power changes because of remifentanil have been described previously, including an increase in α frequency band and a decrease in β frequency bands.[4] Therefore, the reduction of coherence found by Khodayari-Rostamabad *et al.* might be explained by power changes in different frequency bands, which could also lead to spurious alterations of network characteristics.

Several measures of connectivity have been developed to overcome this problem,[2] including the imaginary coherence and the phase lag index. These measures are relatively insensitive to volume conduction and field spread, because they discard zero phase lags.[3] The phase lag index has already been