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Letters to the Editor

The Protective Effect of Vitamins A and C on Endotoxin-Induced Oxidative Renal Tissue Damage in Rats

To the Editor

Kanter et al. (2005) reported a highly interesting series of experiments in which they examined the effect of intraperitoneal injection of vitamins (Vit) A and C against rat endotoxemia. The statistical analysis of their results is, however, inappropriate.

Kanter had a 2 × 2 factorial design with four groups: 0) control, 1) Vit A, 2) Vit C, and 3) both Vit A and Vit C, but they do not analyze the results as would be appropriate for a 2 × 2 design (McAlister et al. 2003). If there is no interaction between Vits A and C, the estimation of Vit A effect should be based on the comparison between groups 1 + 3 (Vit A groups combined) and 0 + 2 (no-Vit A groups combined), and similarly with Vit C. If there is interaction between the treatments, the estimation of Vit A effect should be based on the comparison between groups 1 and 0, and the Vit C effect on the comparison between groups 2 and 0 (McAlister et al. 2003). Also, Kanter focuses on "*p*" values, even though the use of the "*p*" value as the only way to evaluate a potential treatment effect has been strongly discouraged and the use of confidence intervals (CI) has been proposed by several authors (e.g., Gardner and Altman 1986; Braitman 1991).

In their Materials and Methods section Kanter states that "The data were expressed as mean ± S.D." (p. 157). In their Table 4, Kanter et al. report that at the end of the endotoxin experiment the "Mean diameter of glomeruli (μm)" was 43.84 ± 1.23 in untreated, 48.13 ± 1.26 in Vit A treated, and 70.13 ± 2.15 in Vit C treated rats (n = 10 for each group).

From the data in Kanter's Table 4, we can calculate the *t*-test for the difference between the Vit A and C groups and the endotoxin treated control group (for simplicity we calculate as if interaction was established). For the Vit A and control group we find difference of 4.29 μm (S.E. 0.557), which corresponds to t (18 df) = 7.70 and p (2-tail) = 0.0000004. For the Vit C and control group we find difference 26.29 μm (S.E. 0.783), which corresponds to t (18 df) = 33.5 and p = 10 exp (-17).

From Kanter's Table 4 we can also calculate that, compared with the endotoxin treated control group, Vit A increased the mean diameter of glomeruli by 10% (95% CI: 7% to 13%) and Vit C by 60% (95% CI: 56% to 64%), thus both of these estimates are exceptionally accurate. Also, we can calculate that the Vit A + C combination (Table 4: 77.94 ± 3.46 μm) increased the mean diameter by 78% (95% CI: 72% to 83%).

Finally, in the control group not treated with endotoxin the mean diameter of glomeruli was 85.82 μm ± 3.44. Thus without either Vit endotoxin treatment decreased the size of glomeruli by 49% (95% CI: 46% to 52%), but when both Vit were administered the decrease was only 9% (95% CI: 5% to 13%).

In their Results section, Kanter et al. state that "Although Vit C treatments (alone or in combination with Vit A) significantly ($p < 0.05$) increased the diameter of glomeruli in endotoxemic rats, but Vit A treatment did not increase the diameter of glomeruli ($p > 0.05$)." (pp. 158-159). This is misleading when compared with the statistical analyses described above. In the Methods section, Kanter states that they used a nonparametric test, and a small discrepancy in p values would be expected by the different statistical tests (*t*-test used above), however, the divergence is enormous and the *t*-test is well known to be robust so that it gives quite reliable p values also when the distributions substantially differ from the normal distribution.

Thus Kanter's evidence for the benefit of Vits A and C against rat endotoxemia is substantially stronger than they state in their paper.

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References

Braitman, L.E. (1991) Confidence intervals assess both clinical

significance and statistical significance. *Ann. Intern. Med.,* **114**, 515-517.

Gardner, M.J. & Altman, D.G. (1986) Confidence intervals rather than p values: estimation rather than hypothesis testing. *BMJ,* **292**, 746-750.

Kanter, M., Coskun, O., Armutcu, F., Uz, Y.H. & Kizilay, G. (2005) Protective effects of vitamin C, alone or in combination with vitamin A, on endotoxin-induced oxidative renal tissue damage in rats. *Tohoku J. Exp. Med.,* **206**, 155-162.

McAlister, F.A., Straus, S.E., Sackett, D.L. & Altman, D.G. (2003) Analysis and reporting of factorial trials: a systematic review. *JAMA,* **289**, 2545-2553.