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Letter to editor

Comments on the paper "The nephrotoxicity risk in rats subjected to heavy muscle activity" by Gülsen Oner and Selma Cirrik (2009) *Journal of Sports Science and Medicine* 8, 481-488" and response of the authors

Dear Editor-in-Chief,

I have read with great interest the work reported by Gülsen Oner and Selma Cirrik (2009) entitled "The nephrotoxicity risk in rats subjected to heavy muscle activity".

The authors reported that LDL-Cholesterol results of the serum from the rats were calculated by subtracting the HDL-Cholesterol from the Total Cholesterol. I am, however, of the opinion that the formula: LDL-Cholesterol = Total Cholesterol - HDL-Cholesterol should not have been applied by the authors, because the contribution of VLDL-Cholesterol to the Total Cholesterol has not been accounted for. Rats have a high HDL-Cholesterol level with relatively low VLDL-Cholesterol and very low levels of LDL-Cholesterol (Terpstra et al., 1982).

The formula: Total Cholesterol = VLDL-Cholesterol + LDL-Cholesterol + HDL-Cholesterol, is generally accepted as a convenient approximation, because the LDL fraction is heterogenous and contains intermediate-density lipoprotein (IDL), with a density of 1.006-1.019 kg/L, the main LDL, with a density of 1.019-1.063 kg/L and Lipoprotein (a) (Nuack et al., 2002; Rifai and Warnick, 2006).

LDL-Cholesterol can be estimated using both direct and indirect methods. In the indirect methods a number of lipid-related analytes are measured and the results of these analytes are then used in the calculation of the LDL-Cholesterol (Rifai and Warnick, 2006). The two indirect methods of LDL-Cholesterol estimation are beta-quantification and the Friedewald equation.

In the beta-quantification method, ultracentrifugetion is employed to prepare the LDL- Cholesterol plus HDL- Cholesterol fraction. An accurately measured aliquot of the specimen is centrifuged at d 1.006 kg/L for the equivalent of 18 hours at 105,000 x g. The VLDL-Cholesterol and, if present, Chylomicrons and β-VLDL-Cholesterol, will accumulate in a floating layer, with the infranant containing primarily LDL-Cholesterol and HDL-Cholesterol plus any IDL-Cholesterol and Lp(a) that may be present. The infranatant fraction can now be reconstituted to the original volume with 0.15 M NaCl after the supernatant VLDL- and chylomicron-containing fractions have been quantitatively removed. The cholesterol level can then be estimated in the reconstituted infranate. Using a polyanion-divalent cation reagent that provides HDL-cholesterol measurements equivalent to those obtained with the heparin-manganese chloride method, the HDL-containing fraction should be prepared from the ultracentrifugal infranate. The LDL-cholesterol should then be calculated as follows:

[LDL-Cholesterol] = [d >1.006 kg/L Cholesterol] – [HDL-Cholesterol] (Bachorik and Ross, 1995).

In the Friedewald equation, Total Cholesterol, Triglyceride, and HDL-Cholesterol are measured and LDL-Cholesterol is calculated from these measurements using the equation:

[LDL-Cholesterol] mg/dL = [Total-Cholesterol] mg/dL – [HDL-Cholesterol] mg/dL - [Triglyceride] mg/dL /5 (Friedewald et al., 1972). In this formula, the VLDL-cholesterol concentration is estimated by the factor of triglycerides divided by 5.

In conclusion, the formula applied by the authors for the calculation of LDL-Cholesterol would only be appropriate if the beta-quantification method of LDL-Cholesterol estimation was performed. In their study the authors clearly did not perform the beta-quantification method of LDL-Cholesterol estimation and therefore they cannot apply the formula as stated in their article. If the triglycerides were estimated by the authors as part of the rat lipid profile, the Friedewald equation may be applied to estimate LDL-Cholesterol. Alternatively, a direct method for the estimation of LDL-Cholesterol should have been used.

Thys M. Mouton
Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, South Africa

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✉ **Thys M. Mouton**
Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7530, South Africa
E-mail: moutont@cput.ac.za

Authors' response

In our article plasma total cholesterol and HDL cholesterol levels were measured and LDL cholesterol was calculated by subtracting HDL-C from total cholesterol. Meanwhile the contribution of VLDL-C was neglected for two reasons.

1) As mentioned in Dr. Mounon's letter, VLDL-C can be calculated easily using the following equation.
LDL cholesterol = Total cholesterol – HDL cholesterol – (Trigyceride/5)

Like plasma total cholesterol, in our study plasma triglyceride levels were measured and found to be unchanged. Using these triglyceride levels, VLDL-cholesterol levels were estimated as 0.08 ± 0.01 mg/ml and 0.06±0.02 mg/ml in the control and exercising rats respectively. Since the contribution of VLDL-C to LDL-C is limited in rats, these estimated values seemed to us to be negligible. In humans the greater part of VLDL-cholesterol is converted to LDL-Cholesterol, but in rats it has been reported that 80 to 90% of VLDL-C is catabolized by the liver and only 5 to 15% is converted to LDL-C (J Biochem 93(4):1071-77, 1983).

2) It is known that rats are less suitable animals for the study of cholesterol metabolism, although they are commonly used. If we had studied the changes of cholesterol metabolism in exercising rats, the lack of VLDL-Cholesterol values in the studied parameters would have been important. However, we aimed to study the functional characteristics of cholesterol loaded cytoresistant tubule cells of rats subjected to heavy muscle activity. We focused on the change and type of cellular cholesterol and the association of elevated tubule cholesterol with its unaltered plasma levels. Therefore, neither plasma total cholesterol nor HDL cholesterol changed significantly, and the addition of VLDL- cholesterol estimated by dividing unaltered triglyceride levels by 5 to the results seemed to be unnecessary information. However, it would have been more accurate to give our cholesterol results as HDL-Cholesterol and non -HDL cholesterol as do many authors in the literature.

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Gülsen Öner and Selma Cirrik
Akdeniz University, Faculty of Medicine, Department of Physiology, Antalya, Turkey