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Citation Classic

Tissue Lipid Analysis with Enzymatic Reagents

Timothy P. Carr[1*]

Featured Article: Carr TP, Andresen CJ, Rudel LL. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 1993;26:39–42.[2]

The need to measure serum or plasma cholesterol prompted the development of enzyme-based colorimetric assays during the 1970s. These assays eventually replaced the traditional chemical methods. The advantages of the enzymatic procedures were speed and simplicity. On the other hand, because the enzymes naturally work in aqueous environments, the procedures were applicable only to aqueous samples. Enzymatic quantification was not possible for tissue or cellular lipids extracted into organic solvents. Our 1993 report provided several important improvements in this regard. First, we described a procedure for solubilizing tissue lipids in water with the aid of detergent while optimizing enzyme activity. Second, our procedure allowed quantification of all major lipid classes in a single sample preparation. Third, we adapted the procedure to microplates, substantially reducing the volume and expense of enzymatic reagents. Fourth, the microscale approach allowed quantification in very small tissue samples.

At the time our report was published, many laboratories were focusing on atherosclerosis research, so having a fast and reliable method for quantifying major lipid classes in arteries, liver, and other tissues was needed. Quantification of total cholesterol, unesterified (free) cholesterol, triglycerides, and phospholipids was a multistep process in which the lipids were first extracted from tissue samples with organic solvents, followed by separation of the lipid classes with thin-layer chromatography or other chromatographic methods. The separated lipids were then prepared for quantitative analysis by redissolving them in organic solvents. Traditional chemical methods for cholesterol, triglycerides (via glycerol), and phospholipids (via inorganic phosphorus) worked very well with lipids in solvent, but these methods were laborious and required the use of strong acids and other dangerous chemicals. The use of enzymatic reagents for tissue lipid analysis was clearly more desirable, assuming the lipids could be made available to the enzymes without inhibiting activity.

Other laboratories had reported the use of alcohols or detergents to solubilize lipid extracts for cholesterol analysis with enzymatic reagents *(1–3)*. Our initial attempts to adapt these methods were not successful. We found isopropyl alcohol inadequate for solubilizing lipids in a timely manner, even when increased temperatures were applied. Ethanol was somewhat better but caused enzyme inhibition when present in the assay mixture at >20% by volume, thus limiting the range of the assay. We concluded that alcohols were not adequate solubilizing agents, so we turned our attention to the use of biological detergents. Of several detergents tested, we found Triton X-100 to be the most agreeable with the commercial enzymatic kits available at the time. Much of our effort was subsequently devoted to maximizing the range and sensitivity for each enzymatic kit. Since our report was published, we have also used the procedure with enzymatic reagents for phospholipids, free fatty acids, and bile acids, and have not yet encountered an enzymatic kit that could not be used with the procedure.

There are several possible reasons why our procedure has been widely used. Perhaps the most important is that it avoids chromatographic separation of lipid classes before quantification; individual lipid classes can be assayed from a single sample preparation with specific enzymatic reagents. Although greater quantitative and qualitative detail can be achieved with gas chromatography or HPLC, these methods are overkill when only the major lipid classes need to be quantified. The procedure is applicable to a wide variety of biological samples, provided that the lipids can be quantitatively extracted and the solvents completely evaporated before enzymatic analysis. Adapting the procedure to 96-well microplates has the added benefit of analyzing several samples simultaneously on a single plate, which conserves reagents and time. A final benefit is that the procedure is somewhat flexible regarding the amount of tissue extracted and the final volume of the water/detergent/lipid mixture, as long as the amount of detergent is consistent in all samples and the calibrators and the calibration curve are reproducible.

[1] Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE.
* Address correspondence to the author at: Department of Nutrition and Health Sciences, University of Nebraska, 316 Leverton Hall, Lincoln, NE 68506. Fax 402-472-1587; e-mail tcarr2@unl.edu.
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