

## Using TBARS for the Characterization of Ox-LDL

Kalen Biomedical, LLC has received several requests for information about the characterization of our oxidized Low Density Lipoprotein (Ox-LDL) products. We are often asked about TBARS values (thiobarbituric acid reactive substances, primarily measuring certain products from the oxidation of polyunsaturated fatty acids) and how they relate to the total modification of the lipoprotein. We thought it might be beneficial to share some pertinent observations about the value of TBARS in characterizing the extent of the oxidative modification of LDL.

Kalen Biomedical characterizes oxidized lipoprotein products by TBARS, electrophoretic migration, extent of amino group blockage, and protein content.

While TBARS values are widely accepted as evidence of oxidation, they reflect the oxidation of polyunsaturated fatty acids, but provide little information on the total extent of the lipoprotein modification.

The oxidative breakage of covalent bonds in both the lipid and protein moieties of the lipoprotein particle decreases its molecular weight and size. Oxidation of lipids creates more negative charges. The blocking of amino groups, substantially by conjugation with oxidation products of polyunsaturated fatty acids, removes positive charges. All these modifications serve to alter the overall charge density and other properties of the lipoprotein particle.

The parameter that best parallels the total extent of modification of the lipoprotein particle appears to be electrophoretic migration, which is a function of the size of the particle and its electrical charge. Electrophoretic migration is analyzed by agarose gel electrophoresis and expressed here as millimeters of migration beyond that of native LDL.

The generation of TBARS does not correlate with the oxidative modification of LDL as measured by electrophoretic migration. Over the course of oxidation, the TBARS values increase and decrease, while the electrophoretic migration continually increases.

Oxidation High Cu (hrs)	Total TBARS (nmols MDA/mg of protein)	Increase in Migration (mm)
2	62	6
4	76	10
24	47	15

Much of the early TBARS material was not bound to the lipoprotein particle, removed by gel filtration, and recovered in the small molecule fraction (data not shown).

After 18 hours of oxidation, LDL exposed to low levels of copper and isolated from the reaction mixture, expressed considerably more TBARS than did LDL exposed to higher levels.

Copper Level	Ox-LDL associated TBARS (nmoles MDA/ mg of Protein)	Increase in Migration (mm)
Low	78	5
High	16	13

In contrast, but as expected, the LDL exposed to low levels of copper had a smaller increase in electrophoretic migration than did LDL exposed to higher copper levels.

Even after separation of the Ox-LDL particle from the low copper reaction mixture, changes in the lipoprotein can continue and result in further increases in migration.

During manufacture, Kalen stops the oxidation by the addition of EDTA and stabilizes the lipoprotein particle. EDTA chelates the copper and inhibits further initiation of metal catalyzed oxidation.

Oxidation Low Copper	Increase in Migration (mm)	
	Day One	Day Eight
Not Stabilized	5	10
Stabilized	5	5

While EDTA has no effect on the reaction of the TBARS assay with MDA standards, it does have a matrix effect on the TBARS analysis of oxidized lipoproteins.

Oxidation Low Copper	Ox-LDL associated TBARS (nmoles MDA/ mg of protein)
Without EDTA	78
With EDTA	22

The TBARS value for LDL oxidized for 18 hours at low copper concentration drops dramatically when analyzed in the presence of EDTA. The TBARS values of LDL oxidized at higher copper concentrations are low to begin with and change little in the presence of EDTA (data not shown).

In summary, TBARS analyses of oxidized lipoproteins measure one parameter, the lipid oxidation products, either associated with the LDL particle or cleaved from it. The TBARS value is also transitory and depends greatly upon the time that it is measured and the matrix of the sample. TBARS values bear little relationship to the overall lipoprotein modification and concomitant electrophoretic migration. The increase in electrophoretic migration more accurately reflects the overall modification of the LDL particle.

In collaboration with clients evaluating Ox-LDL in biological assays, Kalen has prepared range finding Ox-LDL kits. These kits have proven valuable in determining the optimum conditions and dynamic ranges for the conduct of their studies. For further information about these kits, please e-mail us at [science@kalenbiomed.com](mailto:science@kalenbiomed.com) or call 301-355-6155.