

A simple method to assess the oxidative susceptibility of low density lipoproteins

## ABSTRACT

a simple and useful method is presented for the routine determination of LDL susceptibility to peroxidation in a clinical laboratory.

## INTRODUCTION

Background Atherosclerosis is a pathology that affects many people and may cause their death or disability due to myocardial infarction or strokes. Although the clinical manifestations of the disease have been established, the underlying mechanism of atherogenesis is still unclear. Recent theory points toward the oxidative modification of LDL (LDL-Ox) as one of the major involved processes. Nevertheless, hardly any of the biological effects of LDL-Ox have been tested in vivo. Taking into account the potential clinical importance of the oxidative modification of LDL, many studies have been carried out to quantify their in vitro susceptibility to oxidation. This measurement is thought to correlate with the LDL oxidative susceptibility within the arterial wall. Plasmatic LDLs may be isolated by different methods, which include sequential and density-gradient ultracentrifugation, chromatography, electrophoresis and selective precipitation. Lipid peroxidation is a very complex process that involves the chain reaction of free radicals with polyunsaturated fatty acids. These reactions lead to rearrangements of double bonds in conjugated dienes, hydroperoxide generation, lipid breakdown into lower molecular weight fragments, as well as chemical modifications in the apo B protein. The extent of lipid peroxidation can be estimated by measurement of thiobarbituric reactive substances (TBARS). This method, although nonspecific, is of value in purified systems. TBARS determination mainly measures malondialdehyde (MDA) derived from the hydroperoxidation of unsaturated fatty acids with three or more double bonds. Many studies have been carried out to establish the role of  $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$  in the oxidation of LDL. In biological systems, the reduction of oxygen yields hydrogen peroxide and superoxide radical. The reaction between these two species generates a hydroxyl radical, which is the reactive oxygen species with the shortest half life and highest reactivity. This reaction, which is kinetically slow, can be accelerated by catalytic amounts of iron or copper salts. In the present study we present a simple method which would allow the high-throughput routine evaluation of the oxidative susceptibility of LDLs in the simultaneous presence of  $\text{Cu}^{2+}$  and  $\text{H}_2\text{O}_2$  in the general clinical laboratory. LDLs were isolated by selective precipitation and their oxidative susceptibility was evaluated through the quantitation of TBARS.

## CONCLUSION

A simple method for the in vitro measurement of LDL oxidation susceptibility has been optimized, and applied to a group of healthy subjects and type 2 diabetic patients. This straightforward approach could facilitate the comparison of results obtained from an increased number of general clinical laboratories, and thus allow us to move a step further towards the standardization of a procedure of potential clinical importance.