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Comparison of two methods for radioiodination on the oxidizability properties of low density lipoprotein

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Radiolabeling of low density lipoprotein (LDL) apoB100 with ^{125}I, an oxidative process, is commonly used in lipoprotein investigation. Since 1) LDL is unstable and oxidation-prone, 2) the modification of apoB100 by oxidation increases the negative charge of particles and leads to the uptake of modified LDL through the scavenger receptor pathway, and 3) oxidized LDL is cytotoxic, it is relevant to investigate whether the oxidative stability of LDL is influenced by its labeling with ^{125}I. The aim of this study was to investigate and compare lipid and protein oxidation markers in human LDL after labeling with ^{125}I by two widely adopted methods that use ICl or the chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril as the oxidizing agent. Native LDL served as a common control and sham-iodinated LDL as a handling control for each procedure. The resistance against copper-induced oxidation of ^{125}I-LDL labeled with ICl was similar to that of controls with regard to the lag time and maximal amount of conjugated diene formed, as there were levels of initial conjugated diene, α-tocopherol, and tryptophan. However, radioiodination with the chloramide accelerated the onset of the rapid phase of LDL oxidation due to a drastic depletion of α-tocopherol and increased conjugated diene content. Measurements of copper-induced LDL oxidizability showed enhanced indices of lipid oxidation. The lag time and the time to maximal diene production were 65% and 30% shorter than controls. This was accompanied by a 50% reduced tryptophan fluorescence. The anionic surface charge of the LDL particle increased moderately with both labeling procedures. The results indicate that labeling of LDL with ^{125}I may oxidize lipids and apoB100 to a variable extent, depending on the nature of the iodinating agent. This is why assessment of the oxidizability properties of ^{125}I-labeled LDL is recommended for reliable biological studies.

Key words: Radioiodination, LDL oxidation, Conjugated diene, Lipid peroxidation, ApoB100.

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The use of *in vitro* models is common to evaluate a wide variety of low-density lipoprotein (LDL) biological characteristics, including receptor interaction, clearance and consequences of LDL uptake. In particular, ^{125}I-labeled LDL particles of high specific radioactivity are used in most studies designed to evaluate LDL binding and uptake.

The LDL particle consists of a hydrophobic core of cholesteryl esters and triglycerides, with a surface composed primarily of amphipatic phospholipids, cholesterol and apoB100, which is the ligand for cell surface receptor-mediated uptake. As extensively reported, LDL is extremely labile and oxidation-prone during handling. The oxidative modification of LDL *in vivo* is a free-radical mediated process initiated by polyunsaturated fatty acid peroxidation (7). The subsequent propagation phase triggered by lipid peroxyl radicals is delayed by the presence of oxidizable endogenous antioxidants in the lipoprotein particle. This protecting effect is demonstrated in the typical time-course of copper-promoted LDL oxidation and referred to as the lag phase. The metal-ion-dependent modification of LDL lipids leads to subsequent derivatization of apoB100 lysine and tryptophan residues by reactive lipid-hydroperoxide-derived aldehydes. Finally, apoB100 undergoes oxidative scission and the decomposition phase starts.

As a result of the changes occurring on the LDL particle during the oxidative process, the lipid and the protein components of oxidized LDL differ greatly from those of their native counterparts, and when oxidized to a sufficient extent, apoB100 turns into a more electronegative form capable of interacting with the macrophage scavenger receptor (4). The taking up by the scavenger receptor mechanism results in lipid loading within the cell since this receptor, unlike the LDL receptor, is not downregulated by increased intracellular levels of cholesterol (4). This deregulated LDL uptake mechanism determines the fatty streak intimal lesion in the early phase of atherosclerosis (9). The degree of oxidation of LDL seems to depend upon a wide variety of source-related and experimental conditions. Mildly oxidized LDL might occur naturally as a result of *de novo* oxidation of intrinsic LDL lipids. Such particles contain many lipid peroxidation products that cause modifications in blood, endothelial cells (13, 22, 25) and lipoprotein metabolism (3), and might be physically indistinguishable from the native lipoproteins, except for the expected loss of polyunsaturated fatty acids and antioxidants. ApoB100 is, however, intact and little protein damage has been detected in mildly oxidized LDL (20).

The value of ^{125}I-labeled LDL as tracers for biological studies is based on the assumption that their biological properties are not altered by the iodination procedure or by the radioiodine itself. In this latter regard, many studies have demonstrated that ionizing radiations of low intensity initiate lipid peroxidation in aqueous solutions of fats and oils (i.e., see 16, 18). It has also been reported that after medium- and long-term storages, ^{125}I-labeled LDL showed both increased indices of lipid peroxidation and accelerated fractional catabolic rates in guinea pigs, the modifications being due to the radiated energy (12). Therefore, in addi-

Abbreviations used: LDL, low density lipoprotein; HPLC, high performance liquid chromatography; Iodogen, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (IODO-GEN); PBS, phosphate buffered saline; TBARS, thiobarbituric acid reactive substances.

tion to its antioxidant content, structural and physical elements can affect the oxidative stability of LDL. The methods for the iodination of LDL with ^{125}I differ, among others, in the oxidative power of the oxidizing agent required for conversion of radioactive iodide to iodine and the latter into reactive species that substitute mainly into tyrosine residues of the protein component of the lipoprotein. As the iodination procedure is an oxidation process, undesirable damage of apoB100, either directly by reaction with free radicals or indirectly by lipid peroxidation products, can unfortunately occur during radioiodination, having variable effects on the biological activity of LDL particles.

The method most widely used (15), and its further modifications, for lipoprotein protein radioiodination employs iodine monochloride, ICl, as the oxidizing agent. The chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril, IODO-GEN (Iodogen), either in solution or immobilized, is also currently used for protein radioiodination as it improves labeling yield, reproducibility and technical simplicity (1). Both ICl and Iodogen are documented as mild oxidizing agents in the iodination of proteins. The object of this study was to investigate and compare the oxidizability properties of ^{125}I-labeled LDL, monitored within 24 h of radioiodination, in preparations that had been labeled by either the ICl or the Iodogen method. Our results demonstrate that the nature of the iodinating oxidant dictates whether labeling with ^{125}I may or may not have measurable impact on the oxidizability of LDL. For this reason, the evaluation of protein and lipid oxidation markers of LDL after radioiodination to validate its value in a biological study can be recommended.

Materials and Methods

Isolation and radioiodination of LDL.- Peripheral blood was collected in 1 mmol/L EDTA (sodium salt) from overnight fasted normolipidemic volunteers aged 25-35 years. LDL (1.019-1.063 g/mL) was isolated from fresh plasma by a short-run ultracentrifugation method (27) through a discontinuous density gradient. Further purification was achieved by a second centrifugation step at a density of 1.063 g/mL (120,000 *g* for 18 h at 10 °C). Preparations of LDL were desalted by size-exclusion chromatography using Econo-Pac 10DG columns (BioRad, Hercules, CA), and LDL was recovered in 10 mmol/L phosphate buffered saline (PBS) pH 7.4, containing 10 μmol/L EDTA. The protein concentration of the LDL solution was determined by the method of Lowry *et al.* (14) using bovine serum albumin as a standard. The identity and purity of LDL was confirmed by gradient (3-15%) polyacrylamide gel electrophoresis under nonreducing conditions and staining with Coomassie Brilliant Blue. For each series of experiments, LDL was isolated from the same donor's blood, and processed immediately for radioiodination with ^{125}I using either the iodine monochloride or the Iodogen procedure. In both systems, handling controls (sham-iodinated LDL) were performed, in which the addition of $Na^{125}I$ and the oxidizing reagent were omitted, and are noted as ICl-control and chloramide-control, respectively.

Radioiodination of LDL with ICl was performed after an overnight dialysis of LDL at 4 °C in the dark against 1 mol/L glycine buffer, pH 10, according to a modification (6) of the iodine monochloride method of McFarlane (15). Briefly, 1 mg of LDL protein, 0.15 μg NaI carrier

and 0.5 mCi $Na^{125}I$ (carrier-free $Na^{125}I$, Amersham, UK) in 1 mol/L glycine buffer pH 10 were added to a clamped glass vial, followed by rapid injection of 30 nmol of freshly diluted ICl. The reaction mixture (1 mL) was stirred slowly at 4 °C for 1 min and applied to an Econo-Pac 10DG column to remove free iodine. The ^{125}I-LDL peak was collected in PBS containing 10 µmol/L EDTA from the 3 to 4.5 mL eluate. The specific radioactivity ranged from 20,000 to 70,000 cpm/µg protein. More than 90% of the radioactivity was precipitable by 10% trichloracetic acid.

Radioiodination of LDL with the chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril was performed using IODO-GEN pre-coated iodination tubes (Pierce) according to the manufacturer's instructions for the direct method after an overnight dialysis of LDL at 4 °C in the dark against Tris-HCl buffer, pH 7.5, containing 0.4 mol/L NaCl. Briefly, 1 mg of LDL protein, 0.15 µg NaI carrier and 0.5 mCi $Na^{125}I$ in PBS were added to the IODO-GEN coated tube. The reaction mixture (200 µL) was gently shaken at 1 min intervals at room temperature for 10 min and applied to an Econo-Pac 10-DG column. The ^{125}I-LDL peak was collected in PBS containing 10 µmol/L EDTA from the 3 to 4.5 mL eluate. The specific radioactivity ranged from 40,000 to 90,000 cpm/µg protein. More than 90% of the radioactivity was precipitable by 10% trichloracetic acid. All analyses on LDL samples were done within 24 h of radioiodination.

Analytical procedures.– Thiobarbituric acid-reactive substances (TBARS) in LDL were measured by spectrofluorometry (23). Aliquots of 300 µL of LDL (0.1 mg protein/mL) were mixed with 50 µL EDTA (6.7 mg/mL) and 30 µL of 100% trichloroacetic acid and centrifuged at 2000 *g* for 10 min. Then, 300 µL of the supernatant was incubated with 600 µL of 0.67% thiobarbituric acid in 5% trichloroacetic acid for 15 min in a water bath at 95-100 °C. The fluorescence of the reaction products was measured with wavelengths for excitation at 515 nm and emission at 553 nm.

α-Tocopherol in LDL was determined by reverse-phase high performance liquid chromatography (HPLC) with UV detection at 295 nm using Waters Spherisorb ODS-2 HPLC column (150 x 4.6 mm i.d., 5 µm particle size), as previously described (21). δ-Tocopherol was used as an internal standard. Aliquots of 200 µL LDL (0.2 mg protein/mL) were mixed with 500 µL ethanol containing 2 nmol of δ-tocopherol. Tocopherol was extracted twice with 1 mL n-hexane, and the organic phases were combined and evaporated to dryness under a stream of nitrogen. The extract was dissolved in 20 µL of isopropanol, diluted with 80 µL of the mobile phase, and 50 µL were subjected to HPLC. A methanol/ethanol/isopropanol 65:33.25:1.75 (v/v/v) mixture at a flow rate of 1 mL/min was used as the mobile phase.

Tryptophan residues were measured in LDL (0.1 mg protein/mL) solutions in PBS by fluorescence at an excitation wavelength of 280 nm by the peak emission at 335 nm (11).

The electrophoretic mobility relative to native LDL was determined as an indirect measure of the change in charge on the LDL particle as a consequence of lysine modification. Electrophoresis was carried out in 0.5% agarose gels with 90 mM Tris/borate buffer, pH 8.0, containing 2 mM EDTA. Samples of LDL containing approximately 10 µg of protein in 10%

glycerol were loaded on to the gel, which was run at a constant voltage of 70 V for 2.5 h in a BioRad chamber. The bands were visualized by staining in 0.1% (w/v) Sudan Black in ethanol at room temperature, and destaining of the gel by washing in a mixture of ethanol/water 1:1 (v/v).

Copper-induced LDL oxidation and determination of LDL-oxidizability indices.- The *in vitro* oxidation of LDL was initiated by adding a final concentration of 1.66 µmol/L Cu^{2+}, employed as freshly prepared $CuSO_4$, to LDL solution (62.5 µg protein/mL) in PBS/10 µmol/L EDTA as previously described (8). The degree of lipid peroxidation was determined by monitoring the formation of conjugated dienes at 10-min intervals as the change of the absorbance at 234 nm, using a UVIKON 943-Plus UV-Vis spectrophotometer (Kontron Instruments, Switzerland) equipped with an automatic cell transporter for 10 samples. The mixture was incubated for 3.5 h in the transporter at 37 °C controlled by water circulation. In order to determine the oxidizability of the LDL preparation (8), the increase in absorbance was plotted against time. The initial 234-nm absorbance and the molar absorptivity of conjugated dienes (ε_{234} = 29500 L x mol^{-1} x cm^{-1}) are used to calculate the initial amount of conjugated dienes (mol/g of LDL-protein) present in the sample, referred to as diene (0). The resistance of LDL to oxidation is expressed as the lag time to oxidation, calculated as the interval (min) between the intercept of the tangent to the maximum and minimum rates of oxidation. The maximal rate of conjugated diene formation (mol x min^{-1} x g^{-1} of LDL protein) was expressed by the slope of the rapid propagation phase of the conjugated diene curve. The last index (T(max)) is the time (min) needed to reach maximal absorbance (i.e. produce the maximal amount of conjugated dienes).

Statistical analysis.- Data are expressed as the mean ± SD. The differences between groups were analyzed by the Student's t-test for non paired data. For all comparisons, statistical significance was set at $P \leq 0.05$.

Results and Discussion

In this study we compare the effect of radioiodination with ^{125}I by the ICl or the Iodogen method on LDL oxidizability by measuring the resistance of LDL to oxidation promoted by Cu^{2+} ions and a number of indicators of lipid and protein oxidation. The iodinating agent employed was either iodine monochloride or the chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril, respectively, of which both are documented as mild oxidizing agents in the iodination of proteins. It is worthy to remark that LDL from each donor's blood was either processed immediately for radioiodination or sham-iodination or not processed and used as native LDL.

The formation of conjugated diene structures, which can be measured by absorbance at 234 nm, is both a measure of lipid peroxidation and a reasonable surrogate index of the resistance of LDL to oxidation *ex vivo* (8). Figure 1 shows an experiment representative of the kinetics of conjugated diene formation, in which the three phases of the copper-promoted LDL oxidation are seen. After initiation and propagation, oxidation of native LDL continued for approximately 3 h until the decomposition phase started. All native, the two sham-iodinated and the ICL-labeled LDL samples showed a similar

starting absorbance (Fig. 2A) and a similar pattern of increase in conjugated dienes (Fig. 1). In contrast, the Iodogen-labeled LDL showed an increased absorbance even at the beginning of the oxidation process (Fig. 2A) indicating that oxidation of LDL had occurred in the iodination. When the curve origins were normalized, radioiodination of LDL with the chloramide accelerates oxidation and conjugated diene formation (Fig. 1). The data in table 1 are the mean values of oxidizability parameters calculated for the curves of conjugated diene formation in 4 experiments, each using a different batch of LDL. While no significant differences were observed in the resistance against oxidation between ICl-labeled LDL and ICl-control LDL samples regarding the lag time, the rate of oxidation expressed as the slope of the tangent in the propagation step, and the maximal amount of conjugated diene formed, the ^{125}I-LDL samples labeled with the chloramide had weaker resistance to oxidation, as revealed by a 65% shorter lag phase compared with chloramide-control LDL (Table 1). Since neither maximum rate nor the maximal amount of conjugated diene formed (Table 1) were affected by chloramide labeling, the observed decrease in time necessary to reach maximum diene content in such lipoproteins (Table 1) is probably due to the short lag time.

Besides the increased susceptibility to oxidation by copper, labeling with

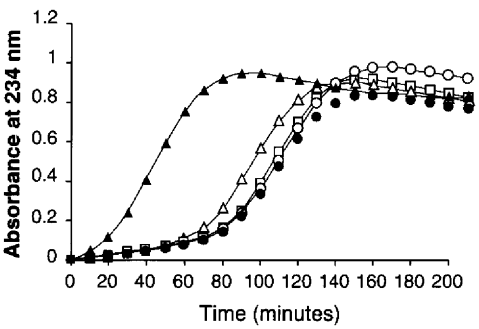

Fig. 1. *Kinetics of copper-induced oxidation of LDL after radioiodination by the ICl or the Iodogen method.*
Native LDL (□), ^{125}I-LDL labeled with ICl (●) or the chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (▲), and their corresponding sham-iodinated controls (○ and △, respectively) were prepared as described in Materials and Methods, and incubated at a final protein concentration of 62.5 μg protein/mL in PBS with 1.66 μM $CuSO_4$ at 37 °C in a spectrophotometer cuvette. Conjugated diene formation was recorded at 10-min intervals by the absorbance at 234 nm and curves are plotted as the change in optical density from the initial value normalized to zero. Data are from a single experiment representative of four performed. From each curve, the four oxidation indices listed in Table 1 were determined.

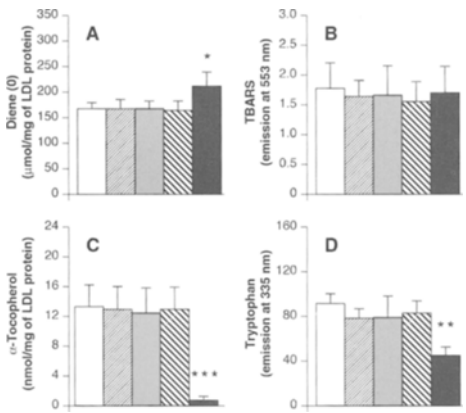

Fig. 2. *Conjugated diene, TBARS, α-tocopherol and tryptophan content in LDL after radioiodination by the ICl or the Iodogen method.*
Native LDL (□), ^{125}I-LDL labeled with ICl (□) or the chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (■), and their corresponding sham-iodinated controls (▨ and ▧, respectively) were prepared as described in Materials and Methods and analyzed for conjugated diene [diene (0)], TBARS, α-tocopherol and tryptophan content. Results are expressed as the mean ± SD of four experiments For each series of experiments, LDL isolated from the same donor's blood was used. Comparisons between Iodogen-labeled ^{125}I-LDL and its control group are indicated by $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$.

Table I. *Oxidizability indices of LDL after radioiodination by the ICl or the Iodogen method.* Experimental conditions were as for Fig. 1. Native LDL, ^{125}I-LDL labeled with ICl or the chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril, and their corresponding sham-iodinated controls were prepared as described in Materials and Methods, and incubated at a final protein concentration of 62.5 µg protein/mL in PBS at 37 °C in the presence of 1.66 µM $CuSO_4$. The formation of conjugated dienes was recorded at 10-min intervals at 234 nm and the oxidation indices were calculated for each curve as described in Materials and Methods. Results are expressed as the mean ± SD of four experiments. For each series of experiments, LDL isolated from the same donor's blood was used. Comparisons between the Iodogen-labeled ^{125}I-LDL group and its control are indicated by *P ≤ 0.05 and **P ≤ 0.01.

Preparation	Lag time (min)	Maximal oxidation rate (mol/min x g LDL protein)	Conjugated diene (max) (mol/g LDL protein)	T (max) (min)
Native LDL	49.2 ± 25.1	0.98 ± 0.16	602 ± 28	112 ± 37
ICl-control LDL	49.5 ± 23.8	0.90 ± 0.32	592 ± 75	122 ± 46
ICl-labeled ^{125}I-LDL	52.8 ± 26.4	0.88 ± 0.15	592 ± 64	122 ± 39
Chloramide-control LDL	42.3 ± 19.2	1.03 ± 0.31	578 ± 55	102 ± 34
Chloramide-labeled ^{125}I-LDL	16.0 ± 5.8**	1.15 ± 0.30	644 ± 68	75 ± 13*

Iodogen provoked LDL oxidation both in the lipid and the apolipoprotein moiety. Although initial conjugated diene content increased by about 30% (Fig. 2A), oxidation was undetectable by TBARS (Fig. 2B), indicating no significantly greater production of the several compounds capable of reacting with thiobarbituric acid. It is well known that lipid peroxidation generates aldehydes, such as 4-hydroxynonenal, which are not measured by the TBARS test and may represent up to a 20% of total aldehydes generated in oxidized LDL (18). The oxidative damage given rise to LDL by labeling with Iodogen is concomitant with a dramatic decrease (from 13.5 to 0.8 nmol/mg of LDL protein) in α-tocopherol content, in the lipoprotein particles (Fig. 2C). It is noteworthy that the native, the two sham-iodinated, and the ICl-labeled LDL samples had levels of vitamin E (a marker of nonoxidized LDL) above 12 nmol/mg protein (Fig. 2C). α-Tocopherol is known to neutralize reactive species in lipidic environments protecting fatty acids against oxidation. In LDL preparations, α-tocopherol and other phenolic antioxidants are consumed gradually along with other endogenous antioxidants contained after the start of *in vitro* oxidation, and thus extend the lag phase of oxidation. When consumed, the propagation step starts without affecting the rate of oxidation. The loss of α-tocopherol indicates that oxidative damage exceeds the protective capacity and probably is the factor that determines the vulnerability to copper-promoted oxidation of such chloramide-labeled ^{125}I-LDL.

Because LDL is notoriously unstable during handling and physical elements can affect its oxidative stability, we examined the effects of the overall manipulations associated with the two methods and found that no measurable degree of alteration of LDL oxidation parameters was detected, as shown in the sham-iodinated LDL samples. These results are in line with those recently reported (5) showing absence of the effect of dialysis and gel fil-

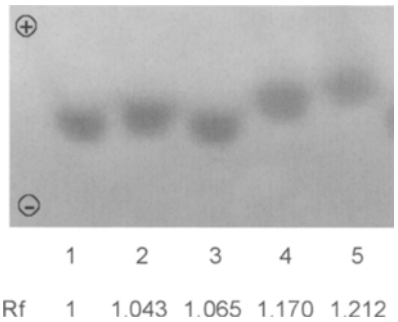

Fig. 3. *Relative electrophoretic mobility of LDL after radioiodination by the ICl or the Iodogen method.* Native LDL (lane 1), ^{125}I-LDL labeled with ICl (lane 4) or the chloramide 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (lane 5) and their corresponding sham-iodinated controls (lanes 2 and 3) were prepared as described in Materials and Methods, and subjected to 0.5% agarose gel electrophoresis in 90 mM Tris/borate buffer, pH 8.0, at 70 V for 2.5 h. LDL bands were visualized by staining in 0.1% Sudan Black in ethanol. The LDL electrophoretic mobility is given as the mean Rf of four analyses performed relative to that of native LDL. The image shows an experiment representative of four performed.

tration on LDL tocopherol and carotenoid content when 10 μmol/L EDTA was included, as in this work.

γ-Radiation emitted by radioiodine may also have oxidative effects. It is well known that radiolysis of water caused by ionizing radiation produces hydroxyl radical and superoxide anion (10) and that both free radicals cause lipid peroxidation and protein modification. In the two methods compared, the same amount of LDL protein (1 mg) was exposed to the same dose of ^{125}I (0.5 mCi). After exposure, reaction mixture was exchanged in the two cases with PBS containing 10 μmol/L EDTA by gel filtration and LDL analysis was performed within 24 hours of labeling. While EDTA addition might not have been sufficient to totally protect LDL against radiation effects (19), the finding that no significant damage was detected in ICl-labeled LDL suggests that the γ-radiation or the covalent attachment of the radioiodine to tyrosine residues did not account for the decreased oxidative stability of chloramide-labeled LDL.

The results above indicate, therefore, that radioiodination by the Iodogen method, but not by the ICl method, generates a modified LDL that represents a form primed for rapid subsequent oxidation. Regarding conjugated diene and α-tocopherol levels, and according to the criteria given in Ref. 20, the chloramide-labeled LDL might be defined as a particle similar to the mildly oxidized LDL. Such a naturally occurring kind of lipoprotein, which is associated with proatherogenic processes including the activation of endothelial cells and platelets (2, 13, 25), should be physically indistinguishable from the native lipoproteins, except for the loss of polyunsaturated fatty acids and antioxidants, with little protein damage (20). However, the protein moiety of Iodogen-labeled LDL presents oxidative modification as revealed by the 50% decrease of the fluorescent emission of tryptophan (Fig. 2D) and the about 20% greater relative electrophoretic mobility (Fig. 3). This latter disturbance, but not the former, was also observed in ICl-labeled LDL (Fig. 3). The stronger the iodination oxidant, the higher the oxidation of amino acid side chains of iodinated proteins (26). In the LDL particle, apoB100 tryptophan residues may be modified by a direct reaction of the aminoacid with free radicals or due to primary oxidative changes in the lipid core. Though Iodogen is documented as mild (1, 19) compared with other oxidants such as chloramine-T, known to cause significant oxidative damage to peptides and proteins (26), a marked derivatization of

tryptophan residues in Iodogen-labeled LDL particles was found (Fig. 2D). A representative analysis of the electrophoretic mobility of native LDL, LDL after radioiodination with ICl or Iodogen and their corresponding controls is shown in Fig. 3. A moderate increase in the relative mobility of LDL was seen after radioiodination by either of the two methods, which indicates that the anionic surface charge of LDL was modified to a more electronegative form. This observation suggests that derivatization of free aminogroups of apoB100 by reactive aldehydes is concomitant to radioiodination, due to γ-radiation and/or iodinating oxidant.

IODO-GEN reagent provides a reproducible, gentle and technically simple means for radioiodination of proteins (1). In our experiments, it was more effective than ICl and labeling of LDL was more reproducible than with ICl (data not shown), but promoted the depletion of α-tocopherol (Fig. 2D) and the formation of conjugated dienes (Fig. 2A). The Iodogen standard procedure requires 10 min to yield a specific labeling 50% superior to that obtained after 1 min exposure using ICl as the oxidant. The longer the exposition time of the LDL particle to the oxidizing agent, the higher the free radical generation and oxidative damage could be. The iodinating oxidant remains with the LDL solution untill separation is achieved. Separation is carried out immediately after labeling with Iodogen, as we have used IODO-GEN pre-coated tubes -a solid phase oxidizing agent-, while chromatographic separation is required after labeling by the ICl method. As a result, similar times of exposition of LDL to the oxidanting agent were registered in both methods. One major difference between these two labeling methods is that chloramide species decompose rapidly forming several radicals (26) whereas ICl does not, so lipid and/or protein peroxidation in LDL may be initiated early by the chloramide. Though efficiency is compromised, it is not unlikely that oxidative modifications of chloramide-labeled LDL are reduced by lowering the time of exposure of the lipoprotein particle to the iodinating oxidant.

The value of ^{125}I-LDL is based on the assumption that radioiodination does not alter the characteristics of LDL. Theoretically, the native LDL particle, as it circulates in the plasma, should contain unmodified apoB100 and no associated peroxides or aldehydes. It is difficult to know whether the newly radioiodinated LDL exhibits a degree of modification that might lead to erroneous estimation of its biological activity. It has been well established that natural modification of apoB100 results from derivatization of apoB residues by products of fatty acid oxidation (24), and when modified to a suficient extent LDL is recognized by the scavenger receptor (4). However, the extent of oxidation of LDL *ex vivo* is not easily controlled and, presumably, biological activity of LDL after labeling with ^{125}I might be unaltered or modified depending on the oxidative power of the iodinating agent together with the methodology used. Studies performed in relation to this topic report the fractional catabolic rate of LDL to be similar after labeling with Iodogen (17) and the specific binding of ^{125}I-LDL to liver membranes to be similar after labeling with Iodogen or iodine monochloride (28). However, ^{125}I-LDL was reported to be more susceptible to pro-oxidant conditions and to show accelerated fractional catabolic rates in guinea pigs after prolonged exposure to γ-radiation (12). These

differences probably reflect differences in sustained oxidative stress associated with the experimental protocols.

In conclusion, the results reported in this paper indicate that in LDL, the kinetics of copper-promoted oxidation, derivatization of free aminogroups of apoB100 and tryptophan and vitamin E contents are markedly affected by radioiodination with Iodogen, whereas only derivatization of free aminogroups is detected by radioiodination with ICl. This work suggests that the vulnerability of LDL to oxidation is not a common property of radioiodination but a prooxidative effect ascribable to the nature of the iodinating oxidant used. For this reason, we recommend the assessment of the oxidizability parameters of ^{125}I-LDL for reliable biological studies.

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El marcaje de la apoB100 de las lipoproteínas de baja densidad (LDL) con ^{125}I es común en la investigación con lipoproteínas. La yodación es un proceso de oxidación. Teniendo en cuenta que 1) las LDL son inestables y se oxidan con facilidad, 2) la oxidación de la apoB100 incrementa la carga negativa de la partícula y provoca su captura por el receptor basurero y 3) las LDL oxidadas son citotóxicas, es relevante investigar si el marcaje con ^{125}I de las LDL afecta a su estabilidad oxidativa. El objetivo de este estudio consiste en analizar y comparar indicadores de oxidación de lípidos y proteínas en LDL humanas marcadas con ^{125}I por dos métodos ampliamente seguidos que emplean como agente oxidante el ICl o la cloramida 1,3,4,6-tetracloro-3α,6α-difenilglucoluril. Las LDL nativas se utilizaron como control común y las LDL yodadas en falso como control procedimental para cada método. La resistencia a la oxidación inducida por cobre de las ^{125}I-LDL marcadas con ICl fue similar a la de las LDL controles referente al tiempo lag, cantidad máxima de dienos formados, así como el contenido inicial de dienos, α-tocoferol y triptófano. Sin embargo, la yodación de las LDL con la cloramida acortó el comienzo de la fase de oxidación rápida por depleción de a-tocoferol y aumento de dienos conjugados. También mostraron índices de oxidación de lípidos superiores (el tiempo lag y el tiempo requerido para la máxima producción de dienos fueron un 65 y 30% más cortos que en sus controles) y una fluorescencia de triptófano 50% inferior. La carga aniónica de la superficie de las LDL aumentó moderadamente con ambos procedimientos de marcaje. Los resultados indican que el marcaje de las LDL con ^{125}I puede oxidar los lípidos y la apoB100 en un grado que depende del agente yodante. Se recomienda, por tanto, valorar los índices de oxidabilidad de las ^{125}I-LDL para validar su uso en estudios biológicos.

Palabras clave: Radioyodación, Oxidación de LDL, Dienos conjugados, Peroxidación lipídica, ApoB100.

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