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# Glucose Accelerates Copper- and Ceruloplasmin-induced Oxidation of Low-density Lipoprotein and Whole Serum

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Glucose at pathophysiological concentrations was able to accelerate copper-induced oxidation of isolated low-density lipoprotein (LDL) and whole serum. The efficiency of glucose was favored under the following circumstances: (a) when LDL oxidation was induced by low copper concentration, (b) when LDL was partly oxidized, i.e. enriched with lipid peroxides. The glucose derivative methyl-α-D-glucoside was ineffective on $Cu^{2+}$-induced LDL oxidation, pointing out the essential role of the reactivity of the aldehydic carbon for the pro-oxidative effect. When LDL oxidation was induced by a peroxyl radical generator, as a model of transition metal independent oxidation, glucose was ineffective. Glucose was found to stimulate oxidation of LDL induced by ceruloplasmin, the major copper-containing protein of human plasma. Thus, glucose accelerated oxidation of LDL induced by both free and protein bound copper. Considering the requirement for catalytically active copper and for the aldehydic carbon, the pro-oxidative effect of glucose is likely to depend on the increased availability of $Cu^{+}$; this is more efficient in decomposing lipid peroxide than $Cu^{2+}$, accounting for acceleration of LDL oxidation. The possible biological relevance of our work is supported by the finding that glucose was able to accelerate oxidation of whole serum, which was assessed by monitoring low-level chemiluminescence associated with lipid peroxidation.

*Keywords*: LDL oxidation; Ceruloplasmin; Glucose; Diabetes mellitus; Serum oxidation

*Abbreviations*: LDL, low-density lipoprotein; CD, conjugated diene hydroperoxide; LL-CL, low-level chemiluminescence; AGE, advanced glycation end products

## INTRODUCTION

Diabetes mellitus and oxidative stress, respectively, represent an established and evolving risk factor for atherosclerosis.[1–3] All lipoprotein classes from human plasma are susceptible to oxidative modification, which elicits a long series of biological effects considered proatherogenic.[4,5] The most important is likely to be the uncontrolled uptake of oxidized LDL by macrophage scavenger receptors in the arterial subendothelial space, eventually leading to foam-cell formation. Enhanced lipoprotein oxidation in diabetes mellitus is supported by several lines of evidence, including reduced resistance against a pro-oxidative stimulus *in vitro*, elevated titers of autoantibodies against oxidized LDL in sera of diabetic patients.[6] Moreover, low concentrations of antioxidant vitamins (notably α-tocopherol)[7] and elevated plasma concentration of lipid peroxidation products, in particular lipid peroxides[8] and $F_2$-isoprostanes have been described in diabetic patients.[9] Interestingly, excretion of $F_2$-isoprostanes in urine was higher in diabetic patients than in age-matched healthy subjects and was reduced after vitamin E supplementation.[10]

A possible molecular mechanism linking diabetes mellitus with atherosclerotic complications is represented by the glycation of proteins.[11,12] However, other mechanisms might be involved, such as mitochondrial dysfunction,[13] reductive stress or

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pseudohypoxia,[14] and glucose autoxidation.[15] In diabetes mellitus, the processes of glycation and oxidation are closely linked and may form a vicious circle, as shown by the following lines of evidence:[11] (a) advanced glycation end products (AGEs) result from free radical-mediated oxidation of reversibly glycated proteins, (b) glycation reactions are associated with release of free radicals, (c) some carbonyl-containing compounds are products of both lipid and carbohydrate oxidation, (d) monocyte-macrophages possess specialized scavenger receptors for AGEs and release free radicals after activation.

It has been recently pointed out that transition metals may participate in the oxidation of glycated products, lipids and carbohydrates.[16] Indeed, many of the glucose-induced oxidative modifications are likely to be mediated by Fenton reactions, which are catalyzed by transition metals;[16] it is at the moment uncertain whether free or complexed metals or both forms are involved. Under certain experimental conditions, ceruloplasmin, the major copper-containing protein of human plasma, can induce oxidative modification of lipoproteins;[17,18] it is considered a model of oxidative damage by copper bound to protein. Several clinical studies have reported an association between ceruloplasmin levels and atherosclerosis.[19,20]

On the basis of all findings described above, it is conceivable that LDL, glucose and metals participate in and contribute to the process of accelerated atherosclerosis, typical of diabetes mellitus. Glucose has been reported to accelerate oxidative modification of LDL induced by copper and iron, by a mechanism which likely involves the $Cu^{2+}$-reducing properties of this sugar.[21,22]

In our study we report that glucose, at pathophysiological concentrations, accelerated the oxidation of isolated LDL and moderately diluted serum by $Cu^{2+}$ and ceruloplasmin. The stimulation of LDL oxidation by glucose was observed under the following experimental conditions: (a) when oxidation was induced by low $Cu^{2+}$ concentrations, (b) when glucose was added late during the lag time of LDL oxidation (thus, LDL was partially oxidized), (c) when moderately diluted serum, containing all lipoprotein classes, as well as water-soluble antioxidants, was used as a substrate for oxidation, (d) when native and intact ceruloplasmin was used as a catalyst of LDL oxidation.

## MATERIALS AND METHODS

### Materials

All reagents were of AR grade and were obtained from either Merck (Darmstadt, Germany) or Sigma (St. Louis, MO, USA). 2,2′-Azobis(2-Amidinopropane) hydrochloride (ABAP) was obtained from Polysciences (Warrington, PA, USA).

### Preparation of LDL

LDL was isolated from plasma of normolipidemic healthy volunteers of both sexes (age 25–35) after overnight fast by ultracentrifugation in a single step discontinuous gradient, as reported by Chung *et al.*[23] LDL was stored in a vial under nitrogen at 4°C in the dark and used within 1 week after preparation. Chemical composition of LDL was normal according to Esterbauer and Ramos.[4]

### LDL Oxidation

Before oxidation, LDL was freed of EDTA by gel filtration. LDL concentration was determined by protein assay using the bicinchoninic acid method; for estimation of molar concentration of LDL the molecular weight of apoB-100 was assumed to be 500,000 Da. LDL, freed of EDTA, was incubated with either $CuSO_4$ (ranging from 0.25 to 2.5 μM), or ABAP (1 mM), or human ceruloplasmin (10 μg/ml) in PBS (NaCl 150 mM, Na-phosphate 10 mM, pH 7.4) at 37°C. Conjugated diene hydroperoxides (CD) were monitored by recording the increase of absorbance at 234 nm (A234 nm) and estimated with a molar absorption coefficient of 29,500 $M^{-1}\,cm^{-1}$.[24] As a kinetic index of LDL oxidation, lag time was determined graphically; maximum rate of oxidation was calculated as the highest value of the first derivative of CD vs. time profile.[24,25] Moreover, LDL oxidation was assessed by monitoring low-level chemiluminescence (LL-CL) associated with lipid peroxidation.[26]

### Chemiluminescence Measurement of Serum Oxidation

Serum from a pool of healthy and fasting donors of both sexes was collected and stored at −80°C for no longer than 3 weeks. It has been shown that ascorbic acid, an important antioxidant of human plasma, was stable for at least 3 weeks in serum stored at −80°C.[27] In thawed serum-pool, the concentration of ascorbic acid and uric acid was assayed by ion-pairing reversed-phase HPLC as described by Motchnik *et al.*,[28] with the difference that a coulometric electrochemical detector was used, equipped with a guard cell operating at +200 mV and a dual analytical cell operating at −200 and +150 mV.[29] The concentration of ascorbic acid and uric acid was 35 ± 3 and 280 ± 20 μM, respectively, in aliquots of the same serum pool, thawed in different days just before performing the experiments. The

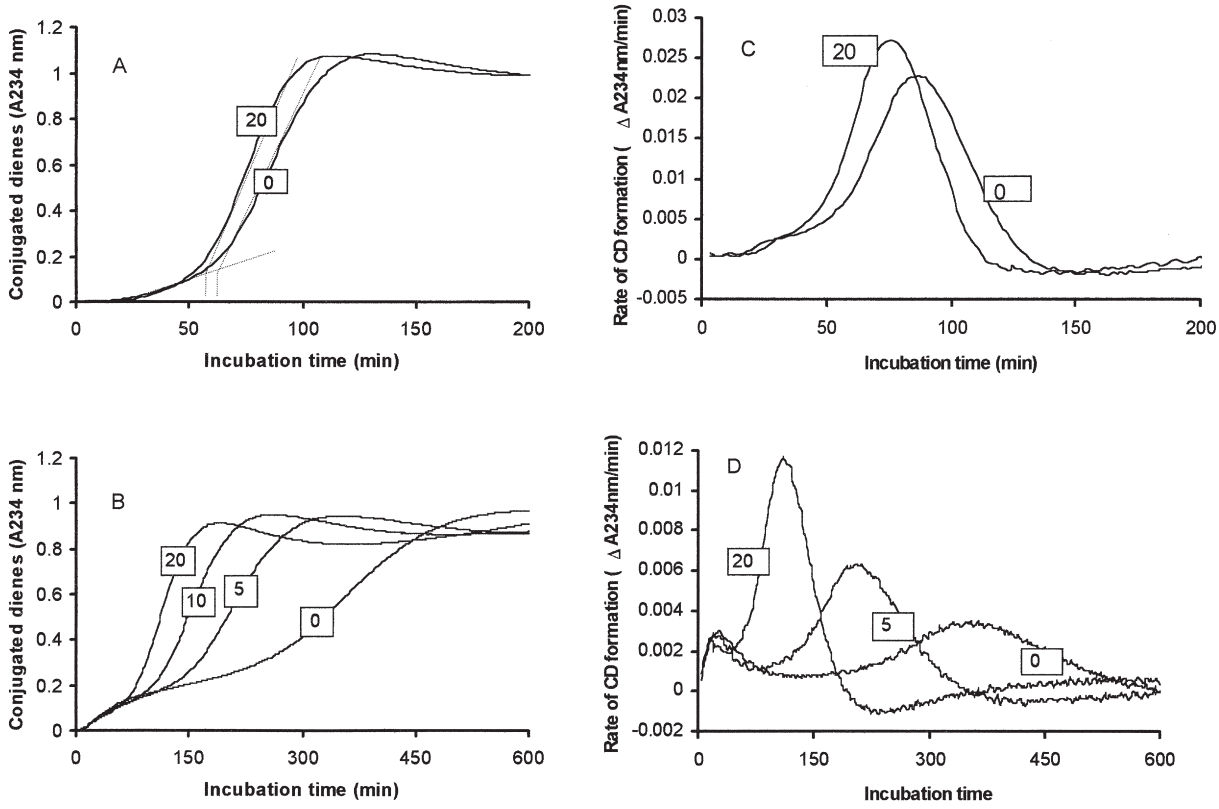


FIGURE 1 Effect of glucose on $Cu^{2+}$-induced LDL oxidation. LDL (40 μg/ml, protein) was incubated in PBS at 37°C either in the absence (0) or in the presence of glucose at the indicated concentrations (in mmol/l). Oxidation was induced by 2.5 (panel A) and 0.25 (panel B) μM $Cu^{2+}$ and was followed by monitoring conjugated dienes, primary products of lipid peroxidation. Lag time determination by graphical method is exemplified in panel A. The profiles of the rate of LDL oxidation, expressed as CD formation/min, at 2.5 and 0.25 μM $Cu^{2+}$ are shown in panel C and D, respectively.



concentration of several lipophilic antioxidants in the thawed serum-pool was assayed by reversed-phase HPLC equipped with a fluorimetric and UV–Vis detector in series, as described by Aebisher *et al.*;[30] the concentration of α- and γ-tocopherol, α- and β-carotene and lycopene was 25 ± 1.5, 1.25 ± 0.3, 0.09 ± 0.02, 0.45 ± 0.03, 0.4 ± 0.03 μM, respectively, in aliquots of the same serum pool. The concentration of glucose in serum pool was assayed with an enzymatic kit from Merck (Darmstadt, Germany) and was 90 ± 3 mg/dl, equivalent to 5 mM. Before experiments, 75 μl of serum were dispensed in a microplate well and diluted with physiologic saline; the total volume of the incubation mixture was 300 μl per well; oxidation was induced by addition of 250 and 500 μM $Cu^{2+}$. LL-CL was measured in a Lucy 1 luminometer (Anthos Labtech Instruments, Salzburg, Austria) equipped with a photon counting photomultiplier (sensitivity ranging from 300 to 700 nm). Integration time for each data point was set to 60 s. The assays were performed at 37°C, in a white plastic microplate. The source of LL-CL is the decay to the ground state of triplet carbonyl compounds produced by recombination of lipid peroxyl radicals to nonradical products.[26]

### Ceruloplasmin Isolation

Ceruloplasmin was isolated according to Farver *et al.*[31] After overnight dialysis against bidistilled water, ceruloplasmin was lyophilized, dissolved in PBS and stored at −20°C. Purity of ceruloplasmin was checked by SDS-PAGE, showing that the intact form of the protein was more than 95%. The ratio of ceruloplasmin specific and aromatic aminoacid residues, A610/A280 nm, another marker of preparation purity, was more than 0.047.[17,18] Ceruloplasmin concentration was determined from its absorbance at 610 nm, with $E^{1\%}_{610} = 0.68$.[17,18]

## RESULTS

### Pro-oxidant Effect of Glucose on $Cu^{2+}$-induced LDL Oxidation

LDL was incubated with copper in the absence and in the presence of increasing pathophysiological concentrations of glucose. At high copper concentration (2.5 μM $Cu^{2+}$, corresponding to a $Cu^{2+}$/LDL molar ratio approximately equal to 31), the kinetics of LDL oxidation, as evaluated from the formation of CD, exhibited a lag and a propagation phase, in

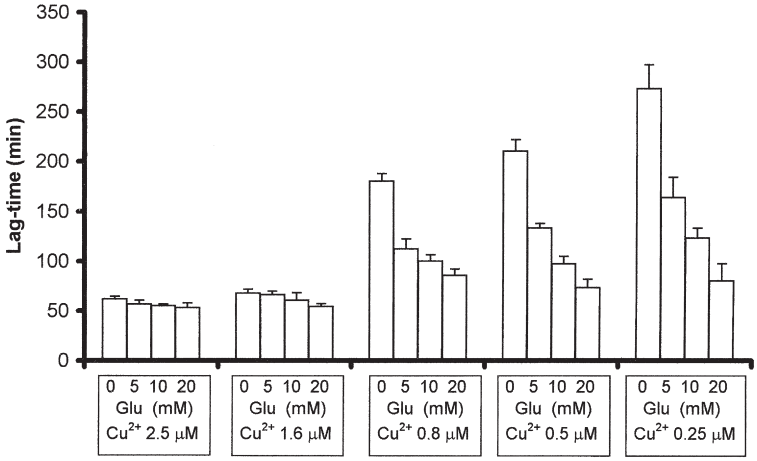


FIGURE 2 Lag time of LDL oxidation at different $Cu^{2+}$ and glucose concentrations. LDL was incubated as described in the legend to Fig. 1 in the presence of $Cu^{2+}$ and glucose at the indicated concentrations. Lag time was determined graphically from CD vs. time profiles. Vertical bars represent the mean + S.D. of five independent experiments.



agreement with many previous studies[4,25] (Fig. 1). Lag time is a useful index of LDL susceptibility to oxidation, which is decreased in the presence of pro-oxidants. Under these experimental conditions, the addition of glucose resulted in a reduction of lag time, which was small and nonsignificant (Fig. 1, panel A). At low copper concentration (0.25 μM $Cu^{2+}$, corresponding to a $Cu^{2+}$/LDL molar ratio approximately equal to 3), the kinetics of LDL oxidation was more complex; it was S-shaped and three phases could be distinguished in the absence of glucose: a preoxidation, a lag and a propagation phase (Fig. 1, panel B). This profile has been often reported when LDL was oxidized at low free radical flux;[32] it is suggestive for an experimental condition when α-tocopherol contributes to the formation of lipid peroxides (tocopherol-mediated peroxidation, following the term introduced at first by Stocker and colleagues[33]). The addition of glucose induced a rapid oxidation of LDL lipids at 0.25 μM $Cu^{2+}$; when 5, 10, 20 mM glucose was added, full oxidation of LDL, indicated by CD maximum concentration, occurred already after 320, 180, 120 min of incubation, respectively, as compared to 600 min in the control (Fig. 1, panel B). The rate of LDL oxidation, expressed as CD formation per minute, is shown in Fig. 1, panel C and D; in the presence of glucose, maximum rate of LDL oxidation was higher and occurred earlier, especially at low $Cu^{2+}$ concentration. Lag time values determined from CD vs. time profiles at various $Cu^{2+}$ and glucose concentrations are reported in Fig. 2; they indicate that the pro-oxidative effect of glucose was concentration dependent and was stronger at lower $Cu^{2+}$ concentrations. Comparable results were obtained when LDL oxidation was monitored with LL-CL (data not shown). Under our experimental conditions, glucose in the absence of $Cu^{2+}$ was unable to induce the formation of conjugated dienes (data not shown).

When LDL oxidation was induced using 1 mM ABAP, a peroxyl radical generator as a model of metal-independent oxidation, glucose was ineffective (data not shown). This result has two implications: (a) glucose does not accelerate LDL oxidation induced by relatively high concentrations of peroxyl radicals (as obtained from 1 mM ABAP decomposition at 37°C), (b) glucose does not possess significant peroxyl radical scavenging effect.

Subsequently, the effect of glucose added during lag time of LDL oxidation was investigated. There are several reasons which justify our interest: (a) the effect of various molecules, all able to reduce $Cu^{2+}$ to $Cu^{+}$, changed from antioxidant to pro-oxidant when added during lag time,[34,35] (b) the flux of initiating radicals during lag time is not constant in the model of $Cu^{2+}$-induced LDL oxidation.[36] On this basis, it is intriguing to investigate which effect is induced by addition of a molecule exhibiting $Cu^{2+}$ reducing ability, like glucose. In the experiment reported in Fig. 3, representative of several, oxidation of LDL was induced by 0.5 μM copper and glucose was included in the incubation mixture at 60 min of incubation; at this time-point of incubation CD hydroperoxide concentration was 2.4 μM (equivalent to 60 nmol CD/mg ApoB-100). Glucose addition induced a rapid oxidation of LDL, as indicated by the onset of propagation phase (Fig. 3A) and the maximum rate of CD formation (Fig. 3B), which was higher and was reached earlier in the presence of glucose. Therefore, partially oxidized LDL appeared particularly susceptible to glucose stimulated $Cu^{2+}$ induced oxidation because a brief time interval was present between glucose addition and the increase of oxidation parameters.

In another set of experiments, either glucose (10 mM) or a mixture glucose–EDTA (10 mM and 100 μM, respectively) were added at later time-points during the lag time. When EDTA was added

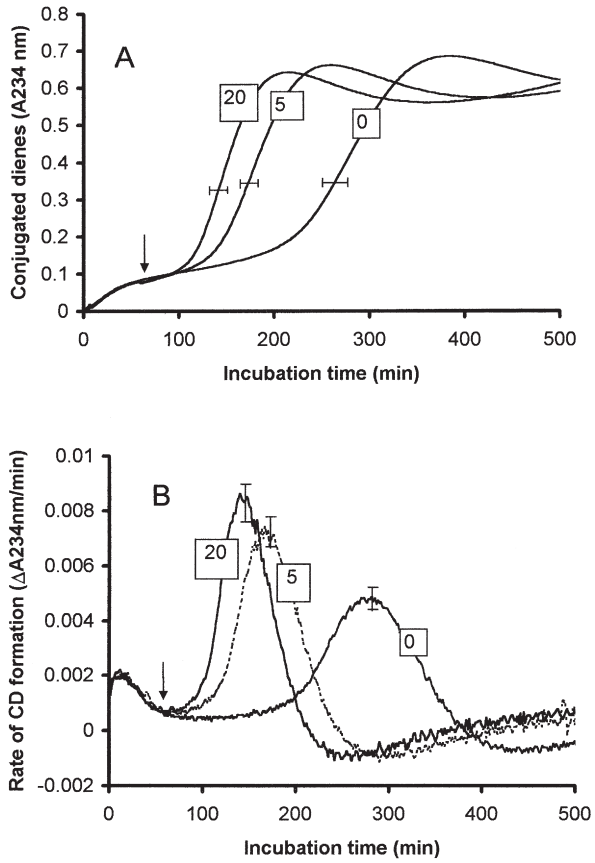


FIGURE 3 Effect of glucose addition to partially oxidized LDL. LDL was incubated as described in the legend to Fig. 1. Oxidation was induced by 0.5 μM $Cu^{2+}$; glucose, at the final concentration of 5 and 20 mM was added at 60 min of incubation (indicated by the arrow). The conjugated diene vs. time profiles and the rate of conjugated diene formation are shown in panel A and B, respectively. In panel A, a horizontal bar indicates the S.D. of the time to reach half-maximum conjugated diene formation determined from independent experiments ($n = 5$). In panel B, a vertical bar indicates the S.D. of the maximum rate of conjugated diene formation.

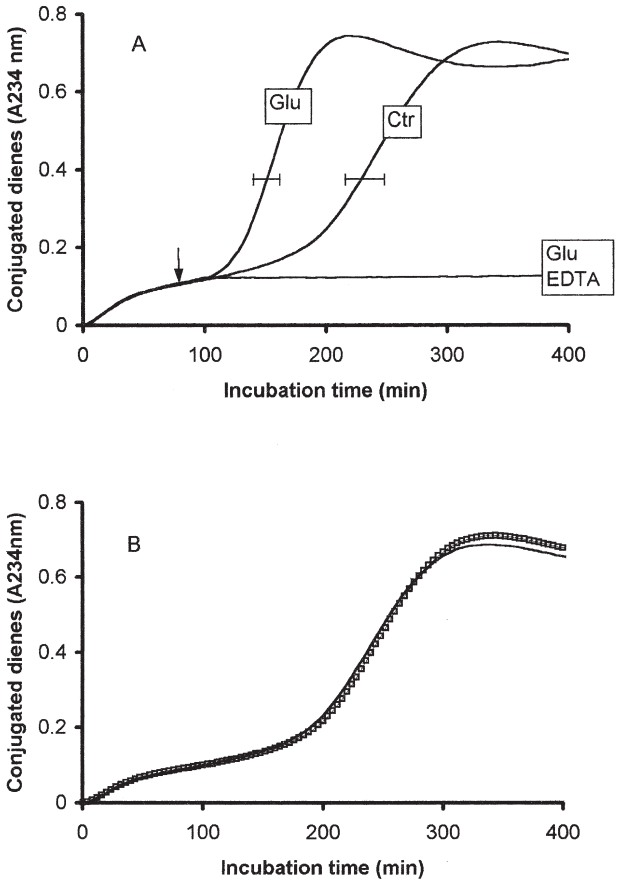


FIGURE 4 In panel A. EDTA blocks the pro-oxidative effect of glucose on LDL oxidation. LDL was incubated as described in the legend to Fig. 1. Oxidation was induced by 0.8 μM $Cu^{2+}$; glucose (Glu), at the final concentration of 10 mM, was added to the incubation mixture at 90 min of incubation (indicated by the arrow) either without or with 100 μM EDTA. For comparison, LDL oxidation profile in the absence of glucose (Ctrl) is shown. As an indication of reproducibility, a horizontal bar indicates the S.D. of the time to reach half-maximum conjugated diene formation determined from independent experiments ($n = 5$). In panel B. Effect of methyl-α-D-glucoside on LDL oxidation. LDL was incubated as described in the legend to Fig. 1. Oxidation was induced by 0.8 μM $Cu^{2+}$ either in the absence or in the presence (line with boxes) of 10 mM methyl-α-D-glucoside.

together with glucose, there was no further increase of CD absorbance, indicating that oxidation was stopped (Fig. 4, panel A). Considering the metal-chelating properties of EDTA, this corroborates that the presence of free copper was a prerequisite for the pro-oxidative effect of glucose.

### Effect of Methyl-α-D-glucoside on $Cu^{2+}$-induced LDL Oxidation

In order to gain insights into the molecular mechanism of the pro-oxidative effect of glucose, a chemically modified hexose, methyl-α-D-glucoside, was included in the LDL oxidation assay. Under experimental conditions similar to those described above, this glucoside was ineffective on copper-induced LDL oxidation (Fig. 4, panel B). This result points out the requirement for a reactive aldehydic carbon for the pro-oxidative effect of glucose in the model of $Cu^{2+}$-induced LDL oxidation.

### Pro-oxidant Effect of Glucose in Diluted Serum

Oxidation of lipoproteins in plasma or serum might be relevant to the physiological situation, but because of analytic difficulties no standard method exists that allows the characterization of lipoprotein oxidizability directly in blood plasma.[37] We evaluated the oxidation of diluted serum induced with $Cu^{2+}$ by recording LL-CL associated with lipid peroxidation. Several copper concentrations and serum dilutions were tested to set up the method, allowing the general conclusion

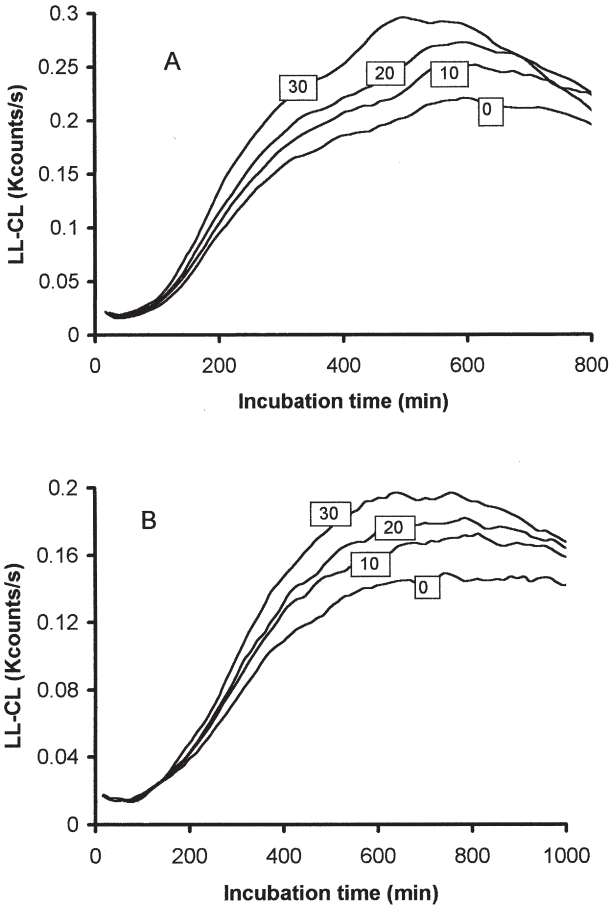


FIGURE 5 Glucose enhanced the oxidation of whole serum. Serum was diluted 4-fold with physiologic saline and was incubated either in the absence (0) or in the presence of exogenously added glucose at the indicated concentrations (in mmol/l); oxidation was induced by 0.5 (panel A) and 0.25 mM $Cu^{2+}$ (panel B). It is worth noting that, considering the basal level of glucose and ascorbic acid, their final concentration was 1.25 mM and 8.7 μM in the control (0), respectively. In the samples containing exogenous glucose, the final concentration is the result of the sum of the basal level of the control and the added amount. Oxidation was monitored by low-level chemiluminescence.

that high $Cu^{2+}$ concentrations resulted in the precipitation of proteins while low $Cu^{2+}$ concentrations were unable to stimulate lipoprotein oxidation, probably due to the copper-chelating ability of serum proteins (notably albumin). Concentrations of 250 and 500 μM $Cu^{2+}$ and a 4-fold dilution of serum were chosen as optimal. The kinetics of $Cu^{2+}$-induced oxidation of diluted serum exhibited a lag phase and a propagation phase, similarly to the oxidation of isolated LDL (at high $Cu^{2+}$ concentration) (Fig. 5, panel A and B). The addition of glucose from the beginning of incubation resulted in an increase of maximum LL-CL, a parameter which was found to be proportional to the square of the oxidation rate.[26] We conclude that glucose exhibited a pro-oxidative effect also in the model of $Cu^{2+}$-induced serum oxidation.

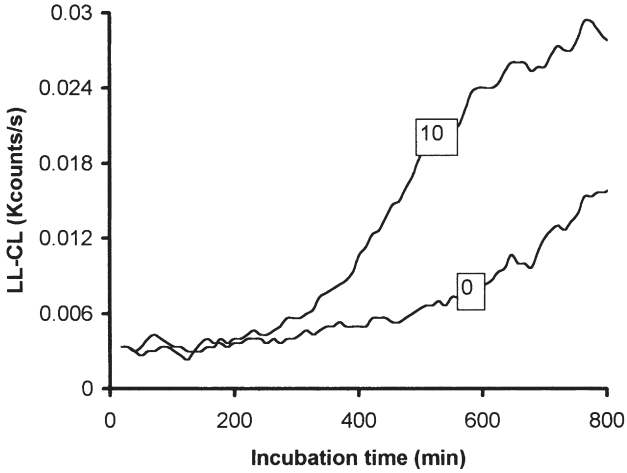


FIGURE 6 Effect of glucose on ceruloplasmin induced LDL oxidation. LDL (150 μg/ml, protein) was incubated in PBS at 37°C in the absence (0) or in the presence of 10 mM glucose (10). Oxidation was induced by 10 μg/ml ceruloplasmin.

### Pro-oxidant Effect of Glucose on Ceruloplasmin-mediated Oxidation of LDL

Ceruloplasmin is the major carrier of copper in plasma; it has been shown that the intact form of the protein is able to oxidize LDL, possibly after binding, by a mechanism which involves a single, redox active copper ion located on the protein surface.[38] Ceruloplasmin, at a concentration of 10 μg/ml (equivalent to $7.5 \times 10^{-2}$ μM), was able to induce oxidation of LDL, as mirrored by an increase of LL-CL. Again, glucose was able to accelerate oxidation (Fig. 6). This result indicates that glucose acted as a stimulator also in a model of protein-bound copper-mediated catalysis of LDL oxidation.

## DISCUSSION

Our investigation provides evidence that glucose, at pathophysiological concentrations, was able to accelerate oxidation of LDL and serum by copper and ceruloplasmin, which can be considered models of free and protein-bound metal catalysts. This finding is to be considered in the light of the recently reappraisal of the role of transition metal catalysis in the pathogenesis of diabetic atherosclerosis, which takes into consideration both forms of the metal.[16]

### Mechanism of the Pro-oxidant Effect of Glucose on Lipoprotein Oxidation

The stimulation of $Cu^{2+}$ mediated oxidation of LDL by glucose showed the following features: (a) it was more evident at low $Cu^{2+}$ concentration, (b) the presence of lipid peroxides was a favoring factor, (c) the presence of both glucose and $Cu^{2+}$ was a

prerequisite, suggesting that a chemical reaction of glucose and $Cu^{2+}$ is responsible for the pro-oxidant effect, d) the aldehydic function of glucose was required, as methyl-α-D-glucoside had no effect on LDL oxidation.

As a conclusion all our data, including the points detailed above, are compatible with the following general reaction scheme:

$$\text{Reaction 1}: \quad \text{Glucose} + Cu^{2+} \rightarrow \text{ox-glucose} + Cu^{+}$$

$$\text{Reaction 2}: \quad Cu^{+} + \text{LOOH} \rightarrow Cu^{2+} + \text{LO}^{\cdot} + OH^{-}$$

$$\text{Reaction 3}: \quad \text{LO}^{\cdot} + \text{LH} \rightarrow \text{LOH} + \text{L}^{\cdot},$$

$$\text{Reaction 4}: \quad \text{L}^{\cdot} + O_2 \rightarrow \text{LOO}^{\cdot},$$

where LOOH is a lipid hydroperoxide, $LO^{\cdot}$ is a lipid alkoxyl radical, LOH is a lipid hydroxide, $L^{\cdot}$ is a lipid carbon centered radical, $LOO^{\cdot}$ is lipid peroxyl radical (the main propagating radical), and $Cu^{2+}$ can be either free or bound to ceruloplasmin.

This set of reactions, to be considered in the light of the well-known metal reducing ability of glucose and of the high capacity of $Cu^{+}$ to decompose lipid peroxide, provides an explanation for the pro-oxidant effect of glucose. A major feature of these reactions is that glucose or its products of oxidation do not possess peroxyl radical scavenging activity, as suggested by its lack of effect on peroxyl radical induced LDL oxidation. To explain the strong pro-oxidant effect of glucose in the presence of low copper concentration, two points must be considered: (a) oxidation of LDL by copper is a saturable process, in the sense that lag time is reaching a minimum value with increasing $Cu^{2+}$/LDL molar ratio[25] (thus the addition of a pro-oxidant agent might have little influence at high $Cu^{2+}$/LDL molar ratio), (b) oxidation of LDL by low copper concentration exhibits a fast "preoxidation" phase with rapid formation of lipid peroxides, as evident in Fig. 1B and in line with a previous report;[32] on this basis, LDL is enriched with (CD), whose decomposition is catalyzed by $Cu^{+}$, formed by glucose oxidation. Both in the model of isolated LDL and whole serum oxidation, we believe that the pro-oxidative effect of glucose is not mainly due to glycation of LDL, because much higher glucose concentration and longer incubation time are required to generate such adducts *in vitro*.[39]

### Possible Physiological Relevance of the Pro-oxidant Effect of Glucose

The physiological bearing of our results is limited by the lack of a satisfactory experimental model to mimic LDL oxidation *in vivo*. In particular, there is scarce knowledge about the oxidative trigger(s) effective *in vivo* and about the radical flux imposed on LDL under physiopathological situations. However, $Cu^{2+}$-induced oxidation of lipoprotein still appears as one of the most used experimental models, being supported by two lines of evidence: (a) oxidized LDL extracted from atherosclerotic lesion resembles $Cu^{2+}$-oxidized LDL as regards the chemical composition and some biological effects,[40] (b) macrophages, the precursors of foam cells, in culture require transition metals for LDL oxidation.[41] It has been recently questioned whether free metals are available in the arterial wall to induce lipid oxidation; this was based mainly on the extremely low level of free copper and iron in plasma and interstitial fluid due to the overwhelming concentration of metal sequestrating plasma proteins[42] and on precise measurement of specific aminoacid oxidation products in early atherosclerotic lesions,[43] which was claimed not to be consistent with free-metal catalyzed process. However, other investigations do support a role for metal induced lipid peroxidation in atherosclerotic lesions: (a) gruel from such lesion contains micromolar amounts of free catalytically active copper and iron,[44] (b) the pattern of chiral fatty acid oxidation products is compatible with nonenzymatic (metal catalyzed) oxidation in advanced atherosclerotic plaque.[45] Moreover, in diabetic macroangiopathy a specific role of Cu-AGE complexes as catalysts of lipid and protein oxidation has been recently proposed.[46]

It is noteworthy that the pro-oxidant activity of ceruloplasmin is enhanced by glucose. Ceruloplasmin is a blue plasma glycoprotein containing seven copper ions.[38] In our experiments, ceruloplasmin was used at a concentration of 0.075 μM, which results in a molar concentration of copper which is included in the range studied by us as free ions. However, only a single copper ion of ceruloplasmin located on the surface of ceruloplasmin[38] appears to be involved in LDL oxidation and is likely to be accessible to the "reducing" glucose which stimulates the oxidative activity. Similar effects of ceruloplasmin in LDL oxidation have been described recently for plasma concentrations of ascorbate and urate.[18] This result is to be linked to the recent evidence that ceruloplasmin may be an independent risk factor for cardiovascular disease.[47,48] However, the data suggesting a link between ceruloplasmin lipoprotein oxidative activity and atherosclerosis have to be reconciled with the observation that albumin is an inhibitor of ceruloplasmin;[17] in this regard, it might be noticed that the ratio between ceruloplasmin and albumin in atherosclerotic patient is likely to be often unbalanced, with an increase of the former[47,48] and a decrease of the latter, as a consequence of the low-grade systemic inflammation associated with atherosclerosis.[49]

The possible physiological relevance of glucose enhancement of LDL oxidation is also supported by the experiments realized with diluted serum as a substrate for oxidation. Two features of lipoprotein oxidation in plasma or serum might be relevant to the physiological situation:[37] (a) all lipoprotein classes undergo oxidative modification, contributing to the formation of peroxidation products, (b) lipoproteins are oxidized in the presence of water-soluble serum antioxidants which are necessarily lost during lipoprotein preparation by ultracentrifugation.

Until now, the use of serum for lipoprotein oxidation measurement was appealing in principle, but was hampered in practice by analytical problems;[37] they were generally referred to the high concentrations of many high- and low-molecular weight compounds which exhibit interference with lipid oxidation measurements. For these reasons most investigations used a high dilution of the serum sample (1/150), raising doubts on the possibility of an interaction between lipid and water-soluble serum antioxidants.[37] The use of a chemiluminescence based method allowed us to circumvent in part this limitation, so that a moderate dilution was applied (1/4).

The extrapolation of our data to the physiological situation, supported by the use of pathophysiological concentrations of glucose and by different oxidation models, suggests the importance of a strict glycemic control in diabetic patients to limit accelerated atherosclerosis and its complications.

As a conclusion, our data as well as a number of recent experimental studies which considered α-tocopherol[23] and related compounds[34] and uric acid,[35] regarding their effect on LDL oxidation, invite to reconsider the generally accepted idea that plasma and interstitial fluid are very rich in antioxidant molecules which would prevent lipoprotein oxidation (which would occur only in sequestered microenvironments). In this context, also the recent finding that ceruloplasmin can oxidize LDL in the presence of physiological concentration of uric and ascorbic acid is relevant.[18] Perhaps more reasonably, we may admit that plasma and interstitial fluid are generally well equipped with antioxidant defenses, but their efficiency is not absolute, depending on the source of oxidative stress and on the conditions of the system under oxidative stress.



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