

# Redox state of albumin is not associated with colloid osmotic pressure

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**Abstract.** Serum albumin exists in oxidized and reduced forms. Although the oxidation of albumin affects some of its functions, the relationship between oxidized albumin and colloid osmotic pressure (COP) remains unclear. The aim of this study was to determine whether there is an association between oxidized albumin and COP. Blood samples from 20 healthy volunteers were divided into two aliquots in order to prepare reduced (n=20) and oxidized albumin samples (n=20). This was achieved by treatment with L-cysteine and a redox-stabilizing agent before and after incubation at 37°C for 24 h. The percentage of oxidized albumin was determined by high-performance liquid chromatography. COP was measured using a colloid osmometer. Reduced and oxidized albumin samples showed 100% of reduced and 100% of oxidized albumin, respectively. There were no significant differences in albumin level and total protein level between the reduced and the oxidized albumin samples. No significant change was seen in COP between the reduced and the oxidized albumin samples (reduced albumin, 17.4±0.2 mmHg; oxidized albumin, 17.3±0.2 mmHg; P=0.465). Therefore, there is no significant difference in COP between reduced and oxidized albumin samples.

## Introduction

Human serum albumin (HSA) is synthesized by hepatocytes and is the most abundant protein in plasma. HSA acts not only as a transporter of various substances, but also as a component of colloid osmotic pressure (COP) (1,2). COP is an important factor that regulates the movement of fluids between intravascular and extravascular spaces (3). Since large plasma proteins cannot easily cross through the capillary walls, their effect on the osmotic pressure of the capillary interiors tends to pull fluid into the capillaries (4). HSA accounts for 80% of plasma COP, therefore it is believed that a decrease in serum albumin levels leads to low COP, which is associated with the development of fluid retention in the interstitial space or so-called edema (5). However, edema cannot always be explained by decreases in the serum albumin level (6).

HSA is divided into two forms according to the redox state of the Cys-34 locus of HSA: reduced albumin (human mercaptalbumin) and oxidized albumin (human non-mercaptalbumin). This has been demonstrated by high-performance liquid chromatographic (HPLC) analysis (7). In healthy adults, approximately 75% of the Cys-34 molecules in albumin contain a free sulfhydryl group (reduced albumin), while approximately 25% (oxidized albumin) form a disulfide with small sulfhydryl compounds, such as another cysteine, homocysteine or glutathione (8). Not only the quantity, but also the quality of albumin have been previously discussed (9,10). Oxidized albumin clearance in the body is more rapid compared to reduced albumin (11). In addition, ligand binding and antioxidant capacity are lower in oxidized than in reduced albumin (12). Thus, the oxidation of HSA is associated with structural and functional changes.

Recently, we reported that oxidized albumin is associated with edema in cirrhosis (13). Although one would think that the ability to synthesize albumin decreases with disease progression, leading to edema in cirrhotic patients, it remains unclear whether the oxidation of albumin directly affects

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*Abbreviations:* COP, colloid osmotic pressure; HPLC, high-performance liquid chromatography; HSA, human serum albumin

*Key words:* oxidized albumin

Table I. Reduced and oxidized albumin percentage of albumin samples treated with redox-stabilizing agent and L-cysteine.

	Reduced samples (n=20)	Oxidized samples (n=20)	P-value
Oxidized albumin (%)	0±0	100±0	<0.01
Reduced albumin (%)	100±0	0±0	<0.01

Table II. Comparison of laboratory data between reduced and oxidized albumin samples.

	Reduced samples (n=20)	Oxidized samples (n=20)	P-value
Total protein (g/dl)	6.30±0.10	6.20±0.10	0.193
Serum albumin (g/dl)	4.06±0.06	4.05±0.08	0.882
Colloid osmotic pressure (mmHg)	17.4±0.20	17.3±0.20	0.465

COP. The aim of this study was to determine whether the redox state of albumin affects COP.

## Materials and methods

**Preparation of reduced and oxidized human serum albumin samples.** Blood samples from 20 healthy adult volunteers (11 females and 9 males) were each divided into two aliquots in order to prepare reduced (n=20) and oxidized (n=20) albumin samples. Redox-stabilizing agent was prepared as previously described (14).

**Reduced albumin samples.** Samples were exposed to 1 mmol/ml L-cysteine (Sigma-Aldrich, St. Louis, MO, USA) and to redox-stabilizing agent simultaneously, and the mixture was incubated at 37°C for 24 h. Thereafter, samples were stored at -20°C until analysis.

**Oxidized albumin samples.** Samples were exposed to 1 mmol/ml L-cysteine and the mixture was incubated at 37°C for 24 h. Thereafter, samples were mixed with the redox-stabilizing agent and stored at -20°C until analysis.

**Laboratory determinations.** Blood samples were taken from the peripheral vein of the subjects while they were in the sitting position. After the oxidation or reduction treatment, plasma albumin levels were measured using nephelometry and plasma total protein levels were measured using Biuret methods (15,16). In untreated healthy human blood samples, the reference value of plasma albumin is 3,900-4,900 mg/dl; for the plasma total protein level, it is 6.7-8.3 g/dl.

**Determination of oxidized and reduced albumin.** HPLC was performed using 5 µl aliquots of each plasma sample and a Shodex Asahipak ES-502N column (Showa Denko, Tokyo, Japan; column temperature 35±0.5°C). The HPLC system consisted of a Model SCL-10Avp system controller, a Model

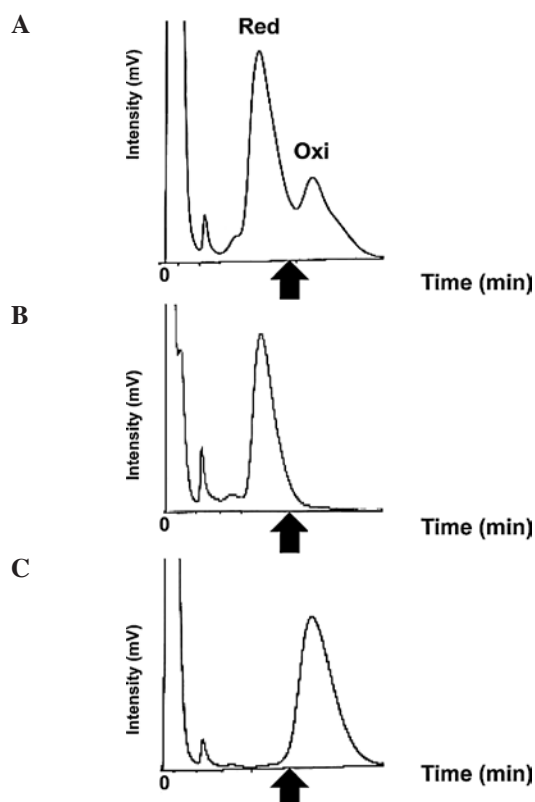


Figure 1. Representative chromatograms of L-cysteine-treated albumin samples. (A) Before treatment (n=4). (B) Reduced albumin sample (n=4). (C) Oxidized albumin sample (n=4). The arrow indicates 20 min past elution. Red, reduced albumin; Oxi, oxidized albumin.

LC-10ATvp double-plunger pump with a Model FCV-10ALvp gradient and a Model SCL-10ADvp autosampler, a Model RF-10AXL fluorescence detector (excitation wavelength 280 nm; emission wavelength 340 nm). All instruments were purchased from Shimadzu Co. (Tokyo, Japan). Elution was carried out with a linear gradient of increasing ethanol concentration 0-5%, in 0.05 M sodium acetate-0.40 M sodium sulfate (pH 4.85) (acetate-sulfate buffer) at a flow rate of 1 ml/min.

**Determination of colloid osmotic pressure.** The colloid osmometer (Colloid 4420®; Wescor, UT, USA) was used to directly measure colloid osmotic pressure (17). To examine the reproducibility of measurements, COP was measured twice for each sample and the values were expressed as the means (Table II).

## Results

**Oxidized albumin percentage of L-cysteine treated samples.** After treatment with L-cysteine + redox-stabilizing agent, the proportion of samples that were reduced albumin became 100%. After treatment with L-cysteine without redox-stabilizing agent, the proportion of samples that were oxidized albumin became 100% (Table I and Fig. 1).

**Albumin levels, total protein levels and colloid osmotic pressure in the reduced and oxidized samples.** Data for the outcome variables are summarized in Table II. There were no significant differences in albumin levels and total protein levels between the reduced and oxidized albumin samples.

Similarly, there was no significant difference in COP between the two groups. Thus, none of the variables differed between reduced and oxidized albumin samples.

## Discussion

In this study, we investigated the association between the oxidation of albumin and COP using 100% reduced and 100% oxidized albumin samples. There was no significant difference in COP between reduced and oxidized albumin samples.

The method for preparing 100% oxidized and 100% reduced albumin samples involved L-cysteine. Since cysteine has an SH residue, treatment with L-cysteine caused the albumin to shift to the reduced form, indicating that cysteine acted as a reductant. Incubation at 37°C for 24 h caused albumin to become oxidized; since cystine (Cys-S-S-Cys) is produced under oxidizing conditions, the result is a shift of the albumin to the oxidized form. With these preparation methods, the only difference between the oxidized and reduced albumin samples was that, in the reduced samples, the cysteine formed disulfide bond with Cys34. Following treatment with L-cysteine, there were no significant differences in the albumin levels or total protein levels between the two groups. However, the concentrations of albumin and total protein decreased compared to the reference values of each parameter. One possible reason is that the samples were diluted by treatment with the redox-stabilizing agent.

We investigated a possible association between the oxidation of albumin and changes in COP. However, no significant difference in COP was detected between the reduced and oxidized albumin samples. COP is the equilibrium pressure exerted on a semi-permeable membrane separating two solutions of differing osmolality. Fluids pass across these membranes, while larger materials, such as proteins (also known as colloids), cannot (18). The molecular mass of human albumin is approximately 66,000, while the molecular mass of L-cysteine is 121.16. It is thought that there is little difference between the molecular mass of oxidized and reduced albumin. COP is dependent on the total concentration of molecules dissolved in a fluid (19). In this study, the concentrations of total protein and albumin did not differ between the reduced and oxidized samples. Therefore, the oxidation of albumin may not have influenced COP. We previously reported that oxidized albumin is associated with edema in cirrhosis (13). In line with this thinking, the indirect influence of albumin oxidation, such as drug binding properties and oxidative stress, may have been related to edema formation (11,20).

In conclusion, we investigated a possible association between the redox state of albumin and COP using 100% oxidized and 100% reduced albumin samples. However, we found that the oxidation of albumin is not associated with changes in COP.

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