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Relation to Vascular Healing and Remodeling

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Abstract—It has been reported that systemic and local redox state may have an important role in the functional and organic changes characterizing the process of vascular response to injury. Carotid endarterectomy to remove atherosclerotic plaque is followed by a long lasting healing and remodeling process that can be carefully followed over time with noninvasive ultrasonography. Plasma vitamin C concentration and native LDL (n-LDL) content in lipid peroxides, vitamin E, β-carotene, and lycopene as well as LDL susceptibility to peroxidation were assessed in 45 patients undergoing elective endarterectomy for internal carotid stenosis, at baseline, 24 hours, 3 and 15 days, and 1 month after surgery. Serial duplex scans were performed in all patients postoperatively and 3, 6, and 12 months. The changes in far wall thickness (FW) and % renarrowing from postoperatively to 12 months were used as remodeling indices. Plasma antioxidant vitamins and lag-phase showed a sharp and significant decrease during the first 24-hours after surgery remaining unchanged until the third day, whereas, an opposite trend was evidenced for n-LDL content in lipid peroxides and serum ceruloplasmin. After the third day all the parameters returned progressively to baseline within one month from endarterectomy. Interestingly, the n-LDL lipid peroxide content, the serum ceruloplasmin and the plasma vitamin C concentration, measured at 24 and 3 days from surgery, were significantly associated to the change in % renarrowing from postoperatively to 12 months. The higher the LDL content in lipid peroxides, the higher the serum level of ceruloplasmin, the lower the plasma content in vitamin C and the higher the % of vessel renarrowing. In conclusion, carotid endarterectomy with atherosclerotic plaque removal is associated with an acute and prolonged increase in systemic oxidative stress that influences vascular healing and late luminal loss. (***Arterioscler Thromb Vasc Biol.*** **1999;19:2659-2665.**)

Key Words: endarterectomy ■ LDL oxidation ■ carotid artery ■ healing ■ vascular remodeling ■ ultrasonography

Carotid endarterectomy to remove atherosclerotic plaque restores blood flow and reduces the risk of cerebral ischemia.[1] This current surgical procedure is followed by a long-lasting healing and remodeling process that in 4% to 22% of patients may induce the development of a recurrent carotid stenosis.[1,2]

Vascular repair and remodeling is a very complex phenomenon that involves a local intense inflammatory response, smooth muscle cell proliferation and migration, and extracellular matrix production and contraction. Thus it may be considered the vascular manifestation of a general biological response to tissue injury, reflecting the systemic wound-healing process.[3–5] In this light it has recently been suggested that systemic and local redox states may have an important role in the functional and organic changes that characterize vessel healing and remodeling after invasive therapeutic procedures.[6–8] In an animal model it has been shown that vascular repair is negatively influenced by a high oxidation state of circulating LDL and benefits from antioxidant vitamin administration.[9] Recently, in humans, vascular surgery such as carotid endarterectomy and coronary angioplasty have been found to be associated with a transient increase in systemic oxidative stress.[10–13] To investigate whether changes in systemic and LDL oxidative status may be associated with vascular healing and remodeling after an invasive therapeutic procedure, we chose the model of the internal carotid artery subjected to elective endarterectomy. This artery does not restenose very often to the extent that symptomatic recurrence needs further intervention,[14,15] but the healing and remodeling process is easy to study over time with noninvasive ultrasonography.[14–16]

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TABLE 1. Clinical Characteristics and Serum Lipids and Lipoproteins at Baseline of the 42 Patients Who Completed the 12-Month Ultrasonographic Follow-Up

Variables	Men	Women
No.	32	10
Age, y	62±11	64±7
Blood pressure (systolic/diastolic)	133±8/83±5	134±7/82±6
Preoperative % stenosis	85±6.5	84±7.2
Duration of intervention, min	83±17	85±15
BMI	26.7±1.8	26.7±1.8
Total cholesterol, mmol/L	6.22±0.7	6.18±0.8
Triglycerides, mmol/L	2.06±0.5	2.02±0.4
HDL cholesterol, mmol/L	1.09±0.12	1.12±0.14
LDL cholesterol, mmol/L	4.18±0.9	4.15±0.8
Apoprotein AI, g/L	1.27±0.14	1.31±0.15
Apoprotein B, g/L	1.29±0.33	1.27±0.31

Methods

Patient Selection
Forty-five patients (35 men, 10 women) admitted to the Vascular Surgery Division of The General Hospital of Chieti (Italy) for elective internal carotid endarterectomy were enrolled in the study.

Selection Criteria
Only patients admitted to the hospital with an indication for monolateral internal carotid endarterectomy were eligible for the study. Inclusion criteria were patients ≥50 years of age (mean age±SD 63±9 years, range 50 to 75 years); ≥70% internal carotid artery stenosis angiographically confirmed; history of transient ischemic attacks or amaurosis fugax; or a single nondebilitating stroke. All women were in the postmenopausal state, but none of them was receiving hormone replacement therapy. Exclusion criteria were current smoking; vitamin supplements or drugs with known antioxidant activity taken within 1 month before surgery; diabetes; previous carotid endarterectomy; impaired consciousness; serious disabling diseases; prosthetic patch angioplasty.

Characteristics of Patients Enrolled
Preoperative angiography demonstrated preocclusive lesions in 22 patients (>90%) and a 71% to 90% stenosis in the remaining 23 (Table 1). All patients were dyslipidemic (Fredrickson type IIa), and 68% were pharmacologically well-controlled hypertensives. Ischemic heart disease was diagnosed in 39% of the patients. None of the patients had significant peripheral vascular disease. All patients were receiving prophylactic treatment with aspirin (100 mg/d) and were following the step II American Heart Association diet throughout the study.

Follow-Up
The patients were followed up by serial duplex scanning performed after surgery and at 3, 6, and 12 months after hospital discharge. Only patients with high-quality B-mode ultrasonographic images of the proximal internal carotid artery were retained in the study. Fasting venous blood samples for determination of biochemical parameters were obtained from all the patients soon before surgery (baseline) and 24 hours, 3 days, 15 days, and 1 month after endarterectomy.

This study was approved by the Ethics Committee of the School of Medicine of the University "Gabriele D'Annunzio" of Chieti (Italy). All patients included in this study gave written informed consent.

Carotid Endarterectomy Procedure
A standard elective endarterectomy procedure was performed under general anesthesia by the same surgeon. Intraluminal shunts were

used for patients with history of stroke, contralateral internal carotid artery stenosis, or occlusion with internal stump pressure <50 mm Hg.

Ultrasonographic Carotid Artery Measurements
Real time, B-mode ultrasound was used to evaluate the internal carotid artery. All examinations were performed by the same trained sonographer with a Hewlett Packard model 77030A ultrasound imaging system equipped with a 7.5-MHz transducer. Subjects were examined in the supine position, with a slight hyperextension of the neck. The internal carotid arteries were examined in a series of cross-sectional scans to select the angle of interrogation that would lead to a perpendicular longitudinal view at the site of the maximal wall thickness. The same interrogation angle was used for each patient during follow-up. Each scan was magnified and recorded on videotape. The indexes of renarrowing were measured from all of these video recordings by the same scanning physician at the end of the study period. The videotapes were read in random order, and the reader was blinded to the name of the patient and the number of the follow-up visit. In all the patients, the new intima-media thickness of the far wall (FW) and the percentage of renarrowing were measured after surgery and at 3, 6, and 12 months after hospital discharge. The FW thickness corresponds to the averaged value obtained on a 1-cm-long longitudinal section starting from the flow divider. The parietal thickness of the far wall was considered the distance from the leading edge of the first echogenic bright line (lumen–new intima interface) to the leading edge of the second echogenic line (wall-adventitia interface).[17] All measurements were made at end-diastole by ECG triggering, with electronic calipers. Percentage of vascular renarrowing was calculated at the point of maximal lumen narrowing by dividing the difference of the reference lumen diameter (RLD) and the minimum lumen diameter (MLD) by the RLD, with the use of the morphometry software of the echo unit.[17] The MLD was considered the distance between the leading edge of the new intima-lumen interface of the near wall and the leading edge of the lumen–new intima interface of the FW.[18] The percent changes in FW thickness and in percent renarrowing (late luminal loss) from after surgery to 12 months of follow-up were used as final measures of the vascular healing and remodeling process. To assess intraobserver variability, 25% of the scans were randomly selected and reexamined by the scanning physician. The intraobserver coefficients of variation for FW thickness and percent renarrowing were 6.9% and 6.4%, respectively.

Laboratory Methods

Serum Lipids and Lipoprotein Assay
Total serum cholesterol and triglycerides were measured by standard enzymatic techniques (Chod-Pap MPR1, Boehringer Mannheim). HDL cholesterol was assessed by immunoturbidimetric technique. The LDL cholesterol (LDL-C) was calculated by Friedewald's formula. Apoprotein AI and apoprotein B were determined by rate nephelometry.[19]

Plasma Vitamin C Determination
Plasma vitamin C was immediately assessed by a spectrophotometric method as previously described.[19]

LDL Isolation and Oxidation
Blood was drawn into test tubes containing EDTA (2.7 mmol/L). The LDL fraction was isolated by single vertical spin ultracentrifugation with a discontinuous NaCl/KBr density gradient as previously reported.[20–22] LDL protein and cholesterol were determined by established methods.[23,24]

LDL (0.2 mg LDL-C/mL) oxidation was triggered by the addition of 5 μmol/L CuSO$_4$ in phosphate-buffered saline, pH 7.4, 37°C. The lag phase preceding the formation of conjugated dienes was calculated as described previously.[25]

Lipid Peroxidation in Native LDL
To improve our measurement of native LDL (n-LDL) content in oxidation products, we used 2 different indirect indexes of lipid peroxidation.

TABLE 2. Changes in Ultrasonographic Indexes Throughout the Study

Parameters	Postop	3 mo	6 mo	12 mo	% (12 mo vs postop)
FW, mm	0.88±0.23	1.16±0.17	1.31±0.26	1.58±0.41*†	+79
% Renarr	17±6.7	26±7.5	31±9	39±13.5†*	+129

Values are mean±SD; data reported refer to the 42 subjects who completed the 12-month follow-up. Postop indicates after surgery; FW, far wall thickness; % Renarr, percentage of vascular renarrowing.

*ANOVA for repeated measures, $P<0.0001$; †Scheffè's test for multiple comparisons, $P<0.001$ (postop vs 12 months).

Lipid peroxidation in n-LDL was assessed by measurement of fluorescent products of lipid peroxidation (FPLPs) and of thiobarbituric acid reactive substances (TBARS). FPLPs essentially reflect the interaction of aldehydic lipid peroxidation products with phospholipids and amino groups of the protein.[21,25–27] The characteristic of these indicators is that they tend to be long-lived and to remain at the sites of oxidative damage.[26] Briefly, an n-LDL sample (1 mL), diluted with PBS to a final protein concentration of 0.5 mg/mL, was mixed with 7 mL chloroform/methanol (2:1 vol/vol) plus water and briefly centrifuged. The lipid-containing phase was removed, dried under a stream of N_2 gas at room temperature, resuspended in chloroform (2.5 mL), and exposed to ultraviolet light. Fluorescence values were estimated spectrofluorometrically at 360-nm excitation and 430-nm emission with the use of a Kontron SFM25 spectrofluorometer calibrated with quinine sulfate. Results were expressed as units of relative fluorescence (URF)/mg LDL-C.

The lipid peroxide content of n-LDL was also evaluated fluorometrically as TBARS.[21] LDL (100 μg protein) was mixed with 1.5 mL of 0.67% thiobarbituric acid and 1.5 mL of 20% trichloroacetic acid containing 1 mg/mL EDTA. After heating at 100°C for 30 minutes, fluorescent reaction products were estimated spectrofluorometrically at 515-nm excitation and 553-nm emission with the use of a Kontron SFM25 spectrofluorometer. Freshly diluted tetramethoxypropane, which yields malondialdehyde (MDA), was used as a standard, and results were expressed as nanomoles of MDA equivalents per milligram of LDL-C (nmol MDA/mg LDL-C).

LDL Antioxidant Determination

LDL content in vitamin E, β-carotene, and lycopene were determined by HPLC as previously reported.[20,21] Vitamins were separated and quantified with the use of a Kontron system 450 equipped with a UV-visible, wavelength-variable Kontron Detector 430. Analysis was performed by isocratic elution. The flow rate was 1.5 mL/min. The mobile phase, consisting of methanol-butanol-water (89.5:5:5.5 vol/vol/vol), was premixed and vacuum-filtered through a 0.45-μm polypropylene membrane filter (Whatman) before use. Autoinjection of 10 μL of organic extract was performed with the use of a Waters autoinjector (model 717 plus Autosampler) refrigerated at 5°C. The analytical column used was a replaceable Partisphere 5 C_{18} cartridge (110 mm×4.7 mm ID, 5-μm particle size, Whatman) protected by a guard cartridge (C_{18}, 5 μm) system and maintained at 45°C. Vitamin E, tocopherol acetate (internal standard), lycopene, and β-carotene were detected by the UV-visible spectrophotometer at different wavelengths programmed for analysis as follows: at 0 minutes, 290 nm; 4.5 minutes, 280 nm; 15 to 22 minutes, 450 nm. Vitamins were expressed as micrograms per milligram of LDL-C (μg/mg LDL-C).

Ceruloplasmin Assay

Serum ceruloplasmin concentration was measured by immunonephelometry with a commercially available kit (QM300, Kallestad Diagnostics Inc) and expressed as milligrams per liter (mg/L). The coefficient of variation of ceruloplasmin content for analytic reproducibility was 2.9%.

Statistical Analysis

Data are reported as mean±SD. An ANOVA for repeated measures followed by a multiple comparison test (Scheffè's test) was performed to test the changes in biochemical and ultrasonographic parameters measured over time. Simple and multiple linear regression analyses were also used.

First, the relation between the biochemical parameters of interest measured at different time points (24 hours, 3 days, 15 days, and 1 month) and the indexes of vascular renarrowing measured as the percent change from after surgery to 12 months was tested by use of simple linear regression analysis. Successively, multiple regression analyses were performed to test the association between changes in FW thickness and percent renarrowing from after surgery to 12 months, and changes over a 24-hour and a 3-day period in those biochemical parameters resulted, at the univariate analysis, as being significantly associated with the ultrasonographic indexes. Thus we created 4 different multiple regression models in which changes in FW thickness and percent renarrowing from after surgery to 12 months were, alternatively, the independent variables, and the changes of the biochemical parameters over the 24-hour period or over the 3-day period represented the dependent variables. All models have been adjusted for potential confounders (levels of the biochemical parameters before surgery, body mass index [BMI], age, sex, ischemic heart disease, systolic blood pressure, percent stenosis before surgery, duration of surgery). Statistical analysis was performed with the STATVIEWS 4.5 software (Abacus Concepts Inc) for the Macintosh Performa 5300 computer.

Results

Baseline clinical characteristics and levels of biochemical parameters of the patients enrolled in the study are reported in Table 1.

Ultrasonographic Measurements

The data are reported in Table 2. The ultrasonographic follow-up was carried out in 42 of the 45 patients enrolled in the study. Three male patients were excluded because of poor ultrasonographic images.

The FW thickness and the percent renarrowing of the internal carotid arteries increased significantly and progressively from after surgery to 12 months. FW increased from 0.88±0.23 to 1.58±0.41 mm (+79%, $P<0.001$) and percent renarrowing from 17±6.7% to 39±13.5% (+129%, $P<0.001$).

Time Course of Biochemical Parameters

Native LDL content in vitamin E, lycopene, β-carotene, the lag phase, and plasma vitamin C concentration showed a sharp and significant decrease during the first 24 hours after surgery; these changes persisted unmodified after 3 days and returned progressively to baseline within 1 month of the surgical operation (Table 3). An opposite trend was seen for lipid peroxide n-LDL content (both indexes) and serum ceruloplasmin, which showed a rapid and significant increase in the first 24 hours, no change until the third day, and subsequent lowering to initial values after 1 month. The highest deviations from the baseline (measured at 24 hours) were n-LDL content in lipid peroxides (FPLPs, +53%,

$P<0.001$; TBARS, +32%, $P<0.001$, respectively) and lyco-

TABLE 3. Biochemical Parameters at Baseline, 24 Hours, 3 Days, 15 Days, and 1 Month After Endarterectomy of the 42 Patients Who Completed 12-Month Ultrasonographic Follow-Up

Variables	Baseline	24 h	3 d	15 d	1 mo	P
FPLPs, URF/mg LDL-C	11.2±1.3	17.2±2.2*	16.8±2.1*	12.0±1.7†	11.7±1.9	0.0001
TBARS, nmol MDA/mg LDL-C	0.68±0.08	0.90±0.10*	0.89±0.10*	0.85±0.13*	0.69±0.08	0.0001
Ceruloplasmin, mg/L	378±65	480±75*	475±80*	425±58*	387±68	0.0001
Lag phase, min	65±6.6	54±5.7*	54±5.6*	59±4.5*	64±6.7	0.0001
Vitamin E, μg/mg LDL-C	3.2±0.5	2.7±0.4*	2.9±0.3*	3.0±0.3‡	3.2±0.4	0.0001
Lycopene, μg/mg LDL-C	0.24±0.04	0.17±0.04*	0.19±0.03*	0.22±0.03‡	0.23±0.04	0.0001
β-carotene, μg/mg LDL-C	0.43±0.1	0.35±0.1*	0.35±0.1*	0.38±0.1‡	0.42±0.1	0.0001
Vitamin C, μmol/L	46±7.2	34±4.6*	35±6.6*	39±4.5*	43±6.4	0.0001

Values are mean±SD. P indicates significance of ANOVA for repeated measures; Scheffè's test for multiple comparisons (vs baseline).

*$P<0.001$, †$P<0.01$, ‡$P<0.05$.

pene (-36%, $P<0.001$), plasma vitamin C (-27%, $P<0.001$), and serum ceruloplasmin ($+24\%$, $P<0.001$). Lower but significant variations were recorded for lag-phase duration (-21%, $P<0.001$), n-LDL content in β-carotene (-21%, $P<0.001$), and vitamin E (-15%, $P<0.001$).

Associations

First, the association between the biochemical variables and the changes in vascular remodeling indexes were tested in a univariate fashion. No statistically significant association was observed between changes (from after surgery to 12 months) in both ultrasonographic indexes of vascular remodeling and baseline clinical characteristics or biochemical parameters (such as ceruloplasmin, n-LDL content in FPLPs or TBARS, plasma vitamin C, and n-LDL content in lipophilic antioxidants). Serum ceruloplasmin, n-LDL content in lipid perox-

ides (FPLPs and TBARS), and plasma vitamin C concentration at 24 hours and 3 days were the only biochemical parameters significantly associated with the changes in FW thickness and in percent vascular renarrowing. The higher the lipid peroxide n-LDL content, the higher the ceruloplasmin serum concentration, the lower the vitamin C plasma level, and the higher the percentage of renarrowing (Figures 1 and 2). FW thickness was also positively related to n-LDL content in lipid peroxides (FPLPs: 24 hours, $r=-0.60$, $P<0.003$; 3 days, $r=-0.45$, $P<0.04$; TBARS: 24 hours, $r=-0.49$, $P<0.03$; 3 days, $r=-0.49$, $P<0.03$, respectively) and serum ceruloplasmin (24 hours, $r=-0.70$, $P<0.005$; 3 days, $r=-0.61$, $P<0.005$, respectively) and inversely related to plasma vitamin C, even if the last relation did not reach statistical significance (24 hours, $r=-0.37$, $P<0.08$; 3 days, $r=-0.40$, $P<0.07$, respectively). On the basis of these
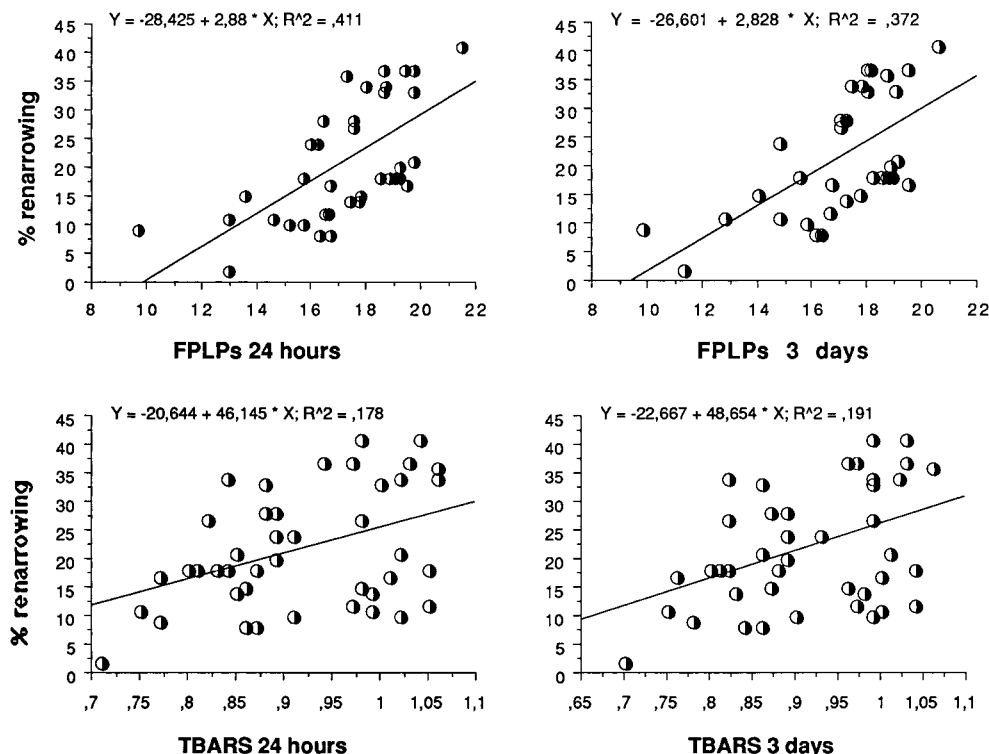

Figure 1. Relation between lipid peroxide n-LDL content measured at 24 hours and 3 days of surgery and percent change of vascular renarrowing at 12 months of follow-up.
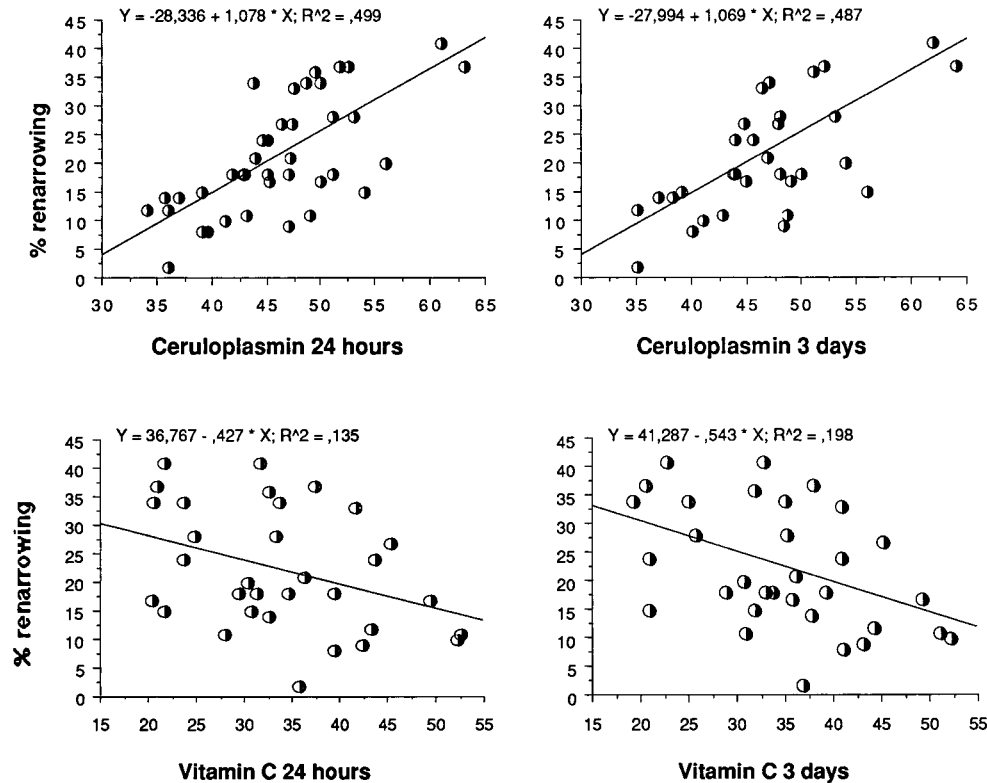
Figure 2. Relation between ceruloplasmin (mg/L) and vitamin C (μmol/L) blood content measured at 24 hours and 3 days of surgery and percent change of vascular renarrowing at 12 months of follow-up.

results, we investigated the association between changes in renarrowing indexes and changes in these oxidative stress parameters over a 24-hour and a 3-day period.

Multiple regression analysis was performed as described in detail in the Methods section. The final models included only FPLPs, vitamin C, and ceruloplasmin (Table 4) and were adjusted for potential confounders (levels of the biochemical parameters before surgery, BMI, age, sex, ischemic heart disease, systolic blood pressure, percent stenosis before surgery, duration of surgery). A higher increase in FPLPs (β=2.372; P<0.0001) and ceruloplasmin (β=1.959; P<0.0001) over a 24-hour period was significantly associated with a higher percentage of vascular renarrowing at 12 months (Table 4). A higher decrease in vitamin C (β=−0.536; P=0.019) over a 24-hour period was significantly associated with a higher percentage of vascular renarrowing at 12 months (Table 4). This

TABLE 4. Results of Multiple Regression Analysis Performed to Test the Relation Between Indexes of Oxidative Stress Measured at 24 Hours and 3 Days and the Percent Change of Vascular Renarrowing From After Surgery to 12 Months After Endarterectomy

Variables	Multiple Regression Model	
	24 h	3 d
FPLPs	2.372, P<0.0001	2.103, P<0.0001
Ceruloplasmin	1.959, P<0.0001	1.433, P<0.0001
Vitamin C	−0.536, P<0.019	−0.714, P=0.0002

FPLPs n-LDL content in fluorescent products of lipid peroxidation. The models have been adjusted for levels of biochemical parameters before surgery, BMI, age, sex, ischemic heart disease, systolic blood pressure, % stenosis before surgery, and duration of surgery.

model was able to explain $\approx$71% (R^2=0.708) of the variation in the change in percent renarrowing over the 12 months. The changes in these oxidative stress parameters over a 3-day period showed a slightly more significant association with the change in percent renarrowing over the 12-month period (R^2=0.712). A weaker association was found when we used FW thickness as the dependent variable both for changes in FPLPs, ceruloplasmin, and vitamin C over a 24-hour period (R^2=0.541) and for changes of the same parameters over a 3-day period (R^2=0.585). This discrepancy between the two ultrasonographic indexes should be mostly attributed to the lesser sensitivity of the FW thickness as a measure of remodeling compared with the percentage of vascular renarrowing. In fact, it is well known that intimal thickness accounts for only a minor proportion of the loss in lumen diameter and that the reduction in circumferential dimension of the entire artery itself constitutes the major cause of late luminal loss.[5,6] On the contrary, the percent renarrowing, which measures the degree of healing and remodeling as the percent reduction of the lumen diameter at the site of maximal narrowing, is expression not only of intima-media thickening but of vascular contraction or expansion and thus is the most appropriate index for the follow-up of lesions over a long period of time.[5,6]

Discussion

In this study we found that patients undergoing elective endarterectomy have a significant increase in systemic oxidative stress. This enhanced and long-lasting systemic oxidant burden was characterized by the concomitant increase in pro-oxidants and decrease in antioxidant blood levels and is mostly attributable to the inflammatory process after surgery and plaque removal.[13] In fact, circulating inflammatory

cells such as monocytes and neutrophils adhere to the site of injury, infiltrate the vessel wall, and produce high amounts of reactive oxygen species (ROS).[7,28–30] Concomitantly, the hepatic production of several proteins is modified with a marked increase in serum level of ceruloplasmin.[31] This increase may be caused in part by monocytes, which have recently been shown to be an important source of ceruloplasmin during the inflammatory response.[32] Ceruloplasmin is a multifunctional protein that behaves as an acute-phase reactant; it tends to be raised after tissue damage or inflammation and it is a marker of severity of the inflammatory process.[33] Moreover, it has recently been shown that intact ceruloplasmin has an important pro-oxidant activity and stimulates cell-mediated and non–cell-mediated LDL oxidation in vitro[33,34] by the Haber-Weiss reactions or by a copper ion–dependent mechanism.[32,33] These two characteristics might explain the association between ceruloplasmin change over time and vascular remodeling. In addition, plaque removal might release iron and copper in catalytic forms that favor free radical reaction.[35] All these events may provide a pro-oxidant environment responsible of a striking increase in local and systemic free radical generation, which affects antioxidant consumption and promotes LDL oxidation. It is generally assumed that the oxidative modification of LDL occurs primarily in the arterial intima because of the many antioxidants present in blood plasma.[36] However, we cannot exclude that in the presence of conditions favoring a systemic pro-oxidant/antioxidant imbalance such as the inflammatory process after surgery with plaque removal, a significant LDL oxidation may occur also in blood plasma.

In this regard, a recent study described a transient increase in systemic oxidant burden immediately after carotid endarterectomy, but it was short-lasting and returned to baseline a few minutes after surgery.[11] In contrast, our results show a more complex and long-lasting phenomenon that appears to influence the process of vascular healing and repairing.

A major finding of our study was that the increased systemic oxidative stress level detected between the first and the third days after surgery was predictive of late luminal loss. In particular, the multivariate analysis showed that changes in lipid peroxide LDL content, ceruloplasmin, and vitamin C within 24 hours and 3 days of surgery were strong predictors of change in percent renarrowing and FW thickness over the 12 months. The high interindividual variability in the oxidative stress response crucially contributes to explain most of the differences observed in the entity of healing, remodeling, and late lumen loss.

The hypothesis that an excess of systemic ROS and increased LDL oxidative modification may influence the process of vascular healing and remodeling is supported by several animal studies.[7–9,30] It has been reported that the presence of excess ROS stimulates intimal thickening, directly promoting migration and proliferation of vascular smooth muscle cells.[30] Oxidative stress also decreases the effective concentration of nitric oxide in the vessel, which has been reported to have a growth-inhibitory effect on vascular smooth muscle cells, to decrease the expression of adhesion molecules for leukocytes, and to inhibit platelet aggregation.[29] Moreover, oxidative cytotoxic products produced by vessel injury may enhance the inflammatory process, impair cellular repair and accelerate cell death, and affect prostaglan-

din and leukotriene synthesis and platelet aggregation.[7,37–40] The severity of the inflammatory stage might predict the severity of wound healing and therefore the total amount of collagen and matrix formation produced as a result of an injury.[3,4,7] Interestingly, a recent study has shown that the acute inflammatory changes peak at 24 hours after endarterectomy and disappear by the fifth day.[3,4] All these data strongly support our findings, which attribute to the increase in systemic oxidative stress an important role in promoting and conditioning vascular healing and renarrowing.

Among antioxidants, only plasma vitamin C was inversely and significantly related to percentage of lumen renarrowing (Figure 2). Our finding could be explained, considering that when free radicals are formed in an aqueous environment, such as plasma, vitamin C acts as the primary defense, whereas vitamin E is consumed only when vitamin C is depleted. Thus in the case of an inflammatory process with a short acute phase as that induced by endarterectomy, vitamin C is likely to be more important than vitamin E in reducing the inflammatory response through early free radical scavenging.[41] In particular, it has recently been reported that during the inflammatory response, myeloperoxidase released from activated neutrophils generates high amounts of an aggressive ROS, mostly hypochlorous acid, which is efficiently and specifically neutralized by vitamin C.[42,43] It has also been shown that vitamin C but not vitamin E or probucol protect, in vivo, endothelium from oxidized LDL–induced leukocyte adhesion and aggregate formation.[44]

A further confirmation of our findings comes from recent studies demonstrating that among antioxidants, only probucol[45,46] and vitamin C,[47] administrated before or immediately after successful percutaneous transluminal coronary angioplasty, were able to significantly attenuate restenosis.

In conclusion, our study shows that surgical procedures to remove atherosclerotic plaque, such as elective carotid endarterectomy, are followed by an acute and long-lasting systemic oxidative stress and that the higher the oxidative stress, the higher the late luminal loss. Moreover, the interindividual variability in the oxidative stress response explains most of the interindividual differences in the entity of vascular remodeling. Finally, our data provide a biological plausibility to the effectiveness of an early treatment with antioxidants, started before any invasive procedure for vascular stenosis, to prevent late renarrowing.

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