Oxidized Low-Density Lipoprotein Cholesterol Is Associated With Decreases in Cardiac Function Independent of Vascular Alterations

Ernst R. Rietzschel, Michel Langlois, Marc L. De Buyzere, Patrick Segers, Dirk De Bacquier, Sofie Bekaeart, Luc Cooman, Patrick Van Oostveldt, Pascal Verdonck, Guy G. De Backer, Thierry C. Gillebert; for the Asklepios Investigators

Abstract—In contrast to the plethora of vasculopathies to which oxidized low-density lipoprotein cholesterol (ox-LDL) can be linked, there are no data linking ox-LDL to myocardial (dys)function in the community. We tested whether ox-LDL, a marker of oxidative stress, was linked to early cardiac structural and functional damage in the general population. The Asklepios Study is a random sample of 2524 male and female volunteers, comparable to the Belgian population between 35 and 55 years free from overt cardiovascular disease. Cardiac morphology, systolic, and early and late diastolic tissue Doppler mitral annulus velocities were recorded during an echocardiography, followed by a vascular examination (carotid and femoral arteries). Serum ox-LDL was measured by sandwich ELISA using the mAb-4E6 monoclonal antibody. Effects of ox-LDL were assessed after adjustment for age, gender, lipid fractions, blood pressure, heart rate, height, weight, glycemia, smoking, and drug treatment. Mean ox-LDL was 96.0±38.9 U/L. After adjustment, increasing ox-LDL levels were associated with a more spherical left ventricular cavity (minor/major axis dimensions; P<0.001) and decreasing diastolic (early diastolic tissue Doppler mitral annulus velocity; P<0.001, more pronounced in women) and systolic function (amplitude of systolic tissue Doppler mitral annulus velocity; P=0.008, more pronounced in men). These results remained unaffected when further adjustments were made for inflammatory markers, lifestyle, or vascular damage (atherosclerosis and arterial stiffening). These results are the first “proof of concept” that ox-LDL impacts cardiac structure and function at a community level, independent of classic risk factors, lifestyle, inflammation, and prevalent vascular damage. Our data suggest that ox-LDL is a risk marker for early ventricular remodelling. However, the effect size in the general population is small. (Hypertension. 2008;52:535-541.)

Key Words: oxidative stress • systolic function • diastolic function • population sciences • oxidized LDL cholesterol • tissue Doppler imaging • cardiac echography

Oxidative stress plays an important role in the pathogenesis of cardiovascular disease, cancer, renal disease, and neurodegeneration.1 It is caused by reactive oxygen species (ROS) and reactive nitrogen species, as well as by redox reactions with substrates able to abstract electrons. Reactive species initiate chain reactions that lead to irreversible chemical changes in lipids (peroxidation) and proteins (oxidation), resulting in cellular dysfunction and cytotoxicity. Basally generated ROS are efficiently detoxified by endogenous enzymatic free radical scavengers. Only when the flux of ROS generated by tissues exceeds the endogenous oxidant defense capacity do deleterious radical-mediated reactions occur.2 The short half-life of reactive species makes them ideal signaling molecules, but it also confounds their measurement in complex biological systems.3 Therefore, much attention has been focused on downstream markers of oxidative stress. Both in vitro and in vivo, low-density lipoprotein (LDL) particles are susceptible to oxidation and peroxidation by all of the causes of oxidative stress outlined above.4,5 Next to the lipid parts, the apolipoprotein B protein part of the LDL molecule might be oxidized as well, with particular involvement of ε-amino groups of lysine residues. The heterogeneous mixture of these modified lipoproteins is usually termed “oxidized LDL (ox-LDL) cholesterol.” During the last decade, several monoclonal antibodies have been generated, each recognizing at least a substantial subset of the whole spectrum of ox-LDL particles, leading to a myriad of new reports on the relation between circulating ox-LDL and cardiovascular pathological processes.1,6–10 Specifically, the
oxidative modification of LDL cholesterol to ox-LDL is thought to be a key initiating step in the development of atherosclerosis. Later ox-LDL acts as a persistent proinflammatory trigger for the progression of atherosclerosis and plaque rupture (atherothrombosis), responsible for the downstream clinical sequelae. Except for certain specific conditions (ischemia-reperfusion injury, adriamycin toxicity, and catecholamine cardiomyopathy), there are very limited data linking oxidative stress to myocardial (dys)function. There are no data in the general population. This is surprising for the following reasons: (1) the plethora of vasculopathies to which ROS and ox-LDL can be linked; (2) the knowledge that coronary endothelial cells, circulating blood cells (eg, leukocytes and platelets), and cardiac myocytes are capable of generating ROS; and (3) experimental data elucidating pathways through which reactive species could induce cardiac damage.\textsuperscript{11,12} Currently there is no confirmation from population studies to support this biologically plausible cardiac damage pathway. We tested whether ox-LDL, a marker of oxidative stress, was linked to early cardiac structural and functional damage in a general population sample. If so, we subsequently tested whether the relation would hold when taking into account markers of vascular damage and concurrent inflammation.

\section*{Methods}
An in-depth description of the Asklepios Study protocol, methodology, and baseline population characteristics has been published.\textsuperscript{13} An expanded Methods section is described in the online supplement available at http://hyper.ahajournals.org.

\section*{Study Population}
We recruited a cohort of 2524 apparently healthy, community-dwelling male and female volunteers aged 35 to 55 years. This random sample is comparable to the Belgian population of that age free from overt cardiovascular disease.\textsuperscript{13} The Ghent University Hospital Ethical Committee approved the study protocol. Baseline data are given in Table 1.

\section*{Participant Examination/Study Components: Overview}
Measurements were as follows (after 15 minute rest, informed consent, and review of questionnaire data): (1) basic clinical data; (2) blood sampling followed by a short rest; (3) echocardiographic examination; and (4) vascular echographic and tonometric measurements. Any study component was always performed by a single observer.

\section*{Cardiac and Vascular Imaging}
The subjects underwent a resting echocardiographic examination and a scan of the left and right carotid and femoral arteries (VIVID 7, GE Vingmed Ultrasound). Left ventricular (LV) internal dimensions were measured at end-diastole (LVIDd) and end-systole (LVIDs) in the minor \textit{minor} and major \textit{major} axes. Sphericity was defined as LVIDd\textsubscript{major} divided by LVIDd\textsubscript{minor}, expressed as a percentage.\textsuperscript{14} Further measurements included the following (methodology described in the online supplement): wall thickness (interventricular septal [IVS] and posterior wall [PW]), LV mass, ejection fraction (EF), systolic (S'), and early (E') and late (A') diastolic mitral annulus pulsed wave tissue Doppler (TDI) velocities, PW Doppler early (E) and late (A) diastolic transmitral flow velocities, and carotid-femoral pulse wave velocity.\textsuperscript{11,13} Intima-media thickness was measured in the bilateral carotid and femoral arteries, which were also carefully scanned for the presence of plaque.
nosis was defined as a carotid or femoral intima-media thickness ≥0.9 mm and/or the presence of carotid or femoral plaque.

**Biochemical Analyses**

Subjects were fasting, had refrained from smoking for ≥6 hours, and were screened for active infection/inflammation. Those with recent or active infection/inflammation returned for their blood sampling after their symptoms had subsided for ≥10 days. Serum oxidized low-density lipoprotein concentration was measured by a sandwich ELISA (Mercodia).\(^7\),\(^5\),\(^6\),\(^16\)

**Statistical Analyses**

Statistical analysis was performed using SPSS for Windows 15.0. We excluded 26 subjects with evidence of acute inflammation (high-sensitive C-reactive protein >20 mg/L). Univariate correlations among ox-LDL, biochemical variables, and echographic indicators were performed using Spearman correlations. Effects of ox-LDL adjusted for confounders were performed with continuous variables whenever possible, using general linear modeling. A P<0.05 was considered to indicate statistical significance.

**Results**

Baseline data are provided in Table 1. Mean ox-LDL was 96.0±38.9 U/L, and median ox-LDL was 91.4 U/L (interquartile range: 68.2 to 118.0 U/L). Ox-LDL was lower in women than in men: 91.5 (quartile range: 68.2 to 118.0 U/L). Ox-LDL was lower in non-smokers than in current or past smokers: 95.0 (quartile range: 69.2 to 117.5 U/L) versus 91.5 (quartile range: 68.2 to 118.0 U/L) (P<0.01) and increased with age (r=0.128; P<0.001). There was also a less pronounced increase in LVIDS\(_{\text{major}}\). This increase in radial dimensions of the LV was not mirrored in the behavior of LV major axis dimensions, which, both at LVIDD\(_{\text{major}}\) and at LVIDS\(_{\text{major}}\), were unaffected in univariate analysis. Ox-LDL was positively correlated with a more spherical LV. Systolic function, when classically addressed as EF, was not affected; however, the amplitude of systolic TDI signals (S') was significantly reduced. Increasing levels of ox-LDL were reflected in decreased diastolic function as evidenced by decreasing E, increasing A, and corresponding lowering of the E/A ratio (r=-0.20; P<0.001). TDI measurements behaved in parallel with decreasing E', increasing A', and lowering of the E'/A' ratio (r=-0.20; P<0.001). Analysis stratified by the presence of hypertension showed that the effects described are similar for subjects with or without hypertension. Figure S1 in the online supplement shows scatterplots for ox-LDL versus sphericity, S' and E'.

**Independent Effects of Ox-LDL on Cardiac Structure and Function**

Effects of ox-LDL were assessed after adjustment for age, gender, total and HDL cholesterol, triglycerides, blood pressure (diastolic and pulse pressure) and heart rate, height, weight, glycemia, current smoking, and drug treatments (antihypertensive and lipid lowering).

### Table 2. Univariate and Multivariate Effects of Ox-LDL on Cardiac Parameters

<table>
<thead>
<tr>
<th>Study Variable</th>
<th>Univariate Effects</th>
<th>Multivariate Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spearman r</td>
<td>P</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>0.06</td>
<td>0.003</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVS, mm</td>
<td>0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PW, mm</td>
<td>0.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVIDD(_{\text{major}}), mm</td>
<td>0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVIDS(_{\text{major}}), mm</td>
<td>0.06</td>
<td>0.002</td>
</tr>
<tr>
<td>LVIDD(_{\text{minor}}), mm</td>
<td>-0.03</td>
<td>0.106</td>
</tr>
<tr>
<td>LVIDS(_{\text{minor}}), mm</td>
<td>-0.01</td>
<td>0.740</td>
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<tr>
<td>Sphericity</td>
<td>0.09</td>
<td>&lt;0.001</td>
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<tr>
<td>LV mass, g</td>
<td>0.18</td>
<td>&lt;0.001</td>
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<tr>
<td>Systolic function</td>
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<tr>
<td>EF, %</td>
<td>0.02</td>
<td>0.387</td>
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<tr>
<td>S', mm/s</td>
<td>-0.05</td>
<td>0.007</td>
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<tr>
<td>Diastolic function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E, cm/s</td>
<td>-0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A, cm/s</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E', mm/s</td>
<td>-0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A', mm/s</td>
<td>0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E/E'</td>
<td>0.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

GLM indicates general linear model; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure. For the multivariate effects analyses, the effects of ox-LDL were assessed after adjustment for age, gender, total and HDL cholesterol, triglycerides, blood pressure (diastolic and pulse pressure) and heart rate, height, weight, glycemia, current smoking, and drug treatments (antihypertensive and lipid lowering).
We tested whether the observed changes on sphericity, S', and E', could be explained by further taking into account lifestyle, inflammation, and vascular damage. Because ox-LDL might be considered a proxy for lifestyle, we overadjusted the above model for uric acid, daily fruit and vegetable intake (grams per day), alcohol intake, educational status, and leisure time physical activity and found no difference (sphericity F = 32.2; P < 0.001; S' F = 7.4; P = 0.006; E' F = 11.5; P = 0.001). Adjustment for inflammatory markers (log-normal transformed high-sensitive C-reactive protein and white blood cell count) did not attenuate the relations (sphericity F = 32.7, P < 0.001; S' F = 7.4, P = 0.007; E' F = 12.5, P < 0.001). Finally we forced measures of vascular damage into the model, hypothesizing that cardiac dysfunction could be secondary to primary vascular damage. Effects on cavity size, S', and E' remained significant when vascular damage parameters were added to the model (presence of atherosclerosis or aortic-femoral pulse wave velocity). Except for a slight attenuation of the impact of ox-LDL on sphericity (F = 27.9; P < 0.001), the other parameters were actually strengthened (S' F = 13.3, P < 0.001; E' F = 15.1; P < 0.001).

In conclusion, the effects of ox-LDL were independent of lifestyle, inflammation, and presence of vascular damage.

**Does Sex Modulate the Effect of Ox-LDL on the Heart?**

The main multivariate analyses were repeated after stratification by sex. The effect on LV structure (no effect on wall thickness or increased sphericity) was similar for both sexes (sphericity: men F = 16.7, P < 0.001; women F = 16.7, P < 0.001). In contrast, the effects of ox-LDL on cardiac function tended to sex specificity. Specifically, in men, increasing levels of ox-LDL seemed to be preferentially associated with loss of systolic function (S': F = 6.0, P = 0.015), with less impairment of diastolic function (E': F = 2.3, P = 0.131). In women, systolic function remained preserved (S': F = 1.4, P = 0.245), but there was a decrease in diastolic function (E': F = 10.7, P < 0.001).

**Discussion**

Our data are the first to demonstrate that ox-LDL impacts cardiac structure and function at the community level. Increasing serum ox-LDL is reflected in a more spherical left ventricle and in decreasing systolic and diastolic function when using sensitive tissue Doppler techniques. These small effects remain significant when taking into account broad adjustments for classic risk factors, lifestyle, and inflammation. Furthermore, the results seem independent from prevalent vascular damage. Significant differences were only observed when using more sensitive and precocious TDI indices instead of classic transmural flow indices of diastolic function or EF as a classic measure of systolic function. As the increased, sphericity was not accompanied by a commensurate increase in LV wall thickness; the net result is increased LV wall stress, which could account for the functional impact. Overall the data indicate that population ox-LDL levels track with important, subtle, and gender-specific cardiac changes but that the effect amplitude (certainly for individual subjects) is small. The present investigation provides the only link so far between ox-LDL and LV shape and function in the general population.

The cardiac changes are indicative of early ventricular remodeling, as seen in the early stages of incident cardiovascular disease. Ox-LDL can, thus, be seen a risk factor (or risk marker, as causality remains unproven) for early ventricular remodeling. The potential added value in the prediction of overt cardiac dysfunction remains to be demonstrated. The sex-specific differences are intriguing. Men and women have a similar increase in LV sphericity. Clinically, it is, therefore, tantalizing that a similar structural stimulus results in a sex-specific functional adaptation. Specifically, increasing levels of ox-LDL seem to be preferentially associated with loss of systolic function in men (with less impairment of diastolic function). In women, systolic function remains better preserved but with decreasing diastolic function. In epidemiology, a pattern of preponderant clinical systolic heart failure in men and a relative preponderance of diastolic heart failure (or heart failure with preserved EF) in women is well documented. Although there are some reports (often from basic science) suggesting that various stressors can have this

**Figure.** Multi-adjusted effects of increasing ox-LDL on cardiac structure and function. Data are percentual changes in cardiac parameters per 50 U/L increase in ox-LDL (the population interquartile range of ox-LDL). Absolute changes were calculated and referenced to the population mean values of the cardiac parameters to give percentual changes to allow comparisons. *Statistically significant independent effects (P < 0.05).
specific behavior, the causal factors for this clinically important sex differential in behavior are largely unknown.\textsuperscript{17–24}

Our results also clearly show the relation between ox-LDL and blood pressure. This effect is expected, because the literature on ox-LDL and atherosclerosis/endothelial dysfunction is robust. The hypothesis that oxidative stress participates in the development of hypertension is attractive, but, because of the cross-sectional nature of our data, we cannot infer any causality. Hopefully longitudinal follow-up data will be able to provide some insights.

Several possible mechanistic explanations for the observed effects on cardiac structure and function can be put forward, as described below.

The first is guilt by association: ox-LDL is a marker of oxidative stress, which in its turn is the cause of radical-mediated myocardial damage. Data on cardiac effects of exposure to ROS in populations are limited. The well-documented effect of oxidative stress in “ischemia-reperfusion injury,” adriamycin toxicity, and catecholamine cardiomyopathy relates to sudden massive oxidative stress levels, far exceeding anything encountered in steady-state humans. However, these extreme conditions provide some relevant mechanistic insights. For an in-depth discussion on these topics, we refer to several excellent reviews.\textsuperscript{1–3,11,12} In brief, cardiac myocytes, coronary endothelial cells, and infiltrating neutrophils can contribute to ROS production, which exerts a direct inhibitory effect on myocardial function through persistent cellular loss of potassium, depletion of high-energy phosphates, elevated intracellular calcium concentration, loss of systolic force development, a progressive increase in diastolic tension, depressed metabolic function, and arrhythmias.\textsuperscript{11} The mechanism underlying the depressed myocardial contractility remains poorly understood, but the combined actions of membrane lipid peroxidation and protein oxidation, with disturbances in calcium homeostasis in a mitochondrial, sarcoplasmatic, and sarcolemmal level, could explain some of the contractile abnormalities.\textsuperscript{25} Data described in congestive heart failure are only indirectly relevant, because markers of oxidative stress reflect the degree of neurohumoral activation and, hence, prognosis in these patients.\textsuperscript{26–28} In heart failure, oxidative stress is secondary to the disease process and, thus, different from our data, which seem to imply an initial role. In congestive heart failure, ox-LDL is correlated with lower EF, severity of the clinical symptoms,\textsuperscript{29} and postinfarction LV volumes\textsuperscript{30} and is a predictor of mortality.\textsuperscript{28}

Second, ox-LDL is a byproduct of exposure to ROS, reactive nitrogen species, and certain redox reactions, which also act as fundamental signal transducers in normal myocardium (expertly reviewed by Hare and Stamler\textsuperscript{31} and by Takimoto and Kass\textsuperscript{32}). Disequilibrium in this system contributes to the transition from LV hypertrophy to heart failure.\textsuperscript{33,34} No population data are available, although experimental data provide a clear framework whereby NO/redox signaling is important in the physiological regulation of cardiac contractility and hypertrophy. ROS stimulation potentially has both adaptive and maladaptive signaling consequences, from a hypertrophic response at low rates of ROS production to fibrosis\textsuperscript{35} and myocyte death at high rates.\textsuperscript{36}

Third, ox-LDL directly causes myocardial damage.\textsuperscript{37} Limited data suggest that incubation of ventricular myocytes with ox-LDL results in intense contractile and electrophysiological changes.\textsuperscript{38}

Finally, oxidatively modified LDL initiates an immune response, which, if autoimmune, could potentially be cardiotoxic. Although there is some evidence that this mechanism could be important in atherosclerosis, no such evidence exists for ox-LDL autoimmune-mediated cardiotoxicity.\textsuperscript{39}

A large part of the variance of ox-LDL remains unexplained. The strongest correlates of ox-LDL are, unsurprisingly, lipid factors, accounting for 20% of the explained variance. In our data set, HDL is one of the strongest and the only negative predictor of ox-LDL. Behavior that increases HDL cholesterol (avoidance of obesity, physical activity, and a prudent diet) is known to be cardioprotective. Whether part of this cardioprotection could be mediated (either directly or indirectly) through ox-LDL is unknown.

Study Limitations
Potential reasons for the observed small effect size are multiple. Ox-LDL might not be a good marker of oxidative stress. Indeed, measuring oxidative stress is notoriously difficult, certainly when population-applicable measures are needed.\textsuperscript{40} Ox-LDL is one of the best studied measures, although there is active controversy regarding both its role as a marker of oxidative stress\textsuperscript{41} and as an independent risk predictor.\textsuperscript{42} Certainly, measurement of a cluster of serum markers for oxidative stress would have strengthened the findings and could have provided additional insights. In a subpopulation of Asklepios participants, the serum ox-LDL test was analytically validated, and values correlated positively with lipid peroxides in plasma as measured by liquid-chromatographic separation of thiobarbituric acid–reactive malondialdehyde.\textsuperscript{16} We could also demonstrate previously that ox-LDL was associated with shortening of peripheral blood leukocyte telomere length, a putative marker of biological aging.\textsuperscript{43} Alternative reasons for the small effect size are that the myocardium might be relatively preserved from, or better defended against, oxidative stress, or that the echographic measures used might not have been sensitive enough. With regard to the former, ongoing research on serum xanthine oxidase activity and/or xanthine oxidase-derived superoxide production from animal or invasive human studies could be valuable. Because xanthine oxidase is an enzyme highly active in cardiac tissue, it might be responsible for increased production of ROS in this tissue particularly. With regard to the latter, the cluster of parameters still significant after multivariate adjustment probably reflects their increased sensitivity compared with more classic parameters. LV sphericity increases with increasing ox-LDL, attributable both to a decreased major axis and to an increased minor axis resulting in a slightly more spherical ventricle. Although difficult to interpret, increased sphericity is sometimes (although far from consistently) observed with aging\textsuperscript{14,44} and is an early marker of LV dysfunction in experimental models of heart failure.\textsuperscript{35,46} In these models, increased sphericity can be prevented by selective matrix metalloproteinase inhibition.\textsuperscript{47} Further studies with novel
functional parameters (strain and strain-rate analysis) are eagerly awaited.

**Perspectives**

Our data are the first “proof of concept” at the community level that ox-LDL impacts cardiac structure and function, in a gender-specific fashion, independent of classic risk factors, lifestyle, inflammation, and prevalent vascular damage. Increasing serum ox-LDL is reflected in a more spherical LV cavity and decreasing diastolic and systolic function when using sensitive tissue Doppler techniques. These results complement experimental evidence that multiple types of cardiac cells can (and probably should) produce reactive species and, conversely, that excess reactive species can damage the contractile machinery of cardiac myocytes. However, the documented effect sizes for such a biologically plausible and allegedly ubiquitous damage pathway are small.

**Acknowledgments**

We thank Frida Brusselmann, Femke Van Hoeke, Linda Packet, and the residents and general practitioners of Erpe-Mere/Nieuwerkerken for their help in completing the study.

**Sources of Funding**

We gratefully acknowledge FWO research grant G042703 (Asklepios Study).

**Disclosures**

None.

**References**


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for the Asklepios Investigators

_Hypertension_. 2008;52:535-541; originally published online July 28, 2008;
doi: 10.1161/HYPERTENSIONAHA.108.114439

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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Data Supplement (unedited) at:
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