Early events in pathogenesis of atherosclerosis

Schematic representation of the initial events of atherogenesis. Native low density Lipoprotein (LDL) becomes trapped in the subendocardial space and can undergo Oxidation by macrophages as well as smooth muscle cells and endothelial cells. Oxidized LDL can stimulate monocyte chemotaxis (A,+), as well as inhibit the Monocyte from exiting through the vascular wall (B,-). Monocytes transform into Macrophages that take up oxidized LDL, becoming foam cells (C). Oxidized LDL can also lead to foam cell necrosis (E) with the release of lysosomal enzymes, and endothelial injury and dysfunction (D). Reproduced with permission from Diaz, MN, Frei, B, Vita, JA, et al. N Engl J Med 1997; 337:408.
Antioxidants and Cardiovascular Diseases

(© 2008 by AstaFactor division of Mera Pharmaceuticals, Inc.)

How does oxidation work in cardiovascular disease?

Atherosclerosis is a condition where the walls of the arteries are damaged and narrowed by deposits of plaque (cholesterol and other fatty substances, calcium, fibrin, and cellular wastes), eventually blocking off the flow of blood. Plaque deposits can result in bleeding (hemorrhage) or formation of a blood clot (thrombus). When hemorrhage or thrombus blocks the flow of blood through the entire artery, a heart attack or a stroke occurs. High blood levels of cholesterol - particularly the cholesterol carried by low-density lipoprotein ("LDL", a protein found in blood) - are associated with an increased risk of atherosclerosis.

Normal LDL in plasma is not oxidized. Oxidation of LDL is believed to contribute to the development of atherosclerosis (Frei 1995). Macrophage cells preferentially take up oxidized LDL, become loaded with lipids, and convert into "foam cells" (Aviram 1996). Foam cells accumulate in fatty streaks, early signs of atherosclerosis. Humans produce auto-antibodies against oxidized LDL, and the levels of such auto-antibodies are higher in patients with atherosclerosis (Frei 1995).

The identification of LDL oxidation as a key event in atherosclerosis suggests that it may be possible to reduce the risk of atherosclerosis by antioxidant supplementation (Ylä-Herttuala 1991). Vitamin E is the major naturally-occurring antioxidant in human lipoproteins (Bowry et al. 1992). Most circulating carotenoids are associated with lipoproteins in plasma (Clevidence and Bieri 1993). The largest fraction of total carotenoids is found in LDL, as evidenced by the typically yellow color of this lipoprotein fraction (Clevidence and Bieri 1993). The largest fraction of hydrocarbon carotenoids (e.g., beta-carotene and lycopene), as well as most vitamin E and other tocopherols, is transported by LDL (Clevidence and Bieri 1993; Goulinet and Chapman 1997; Oshima et al. 1997), suggesting that these compounds in particular may play an important role in preventing oxidative modification of this lipoprotein fraction. The more polar xanthophylls (oxygenated carotenoids such as lutein, zeaxanthin, canthaxanthin, beta-cryptoxanthin, and capsanthin) are distributed more evenly between HDL and LDL (Clevidence and Bieri 1993; Goulinet and Chapman 1997; Oshima et al. 1997). For example, a Japanese study found that ~70% of hydrocarbon carotenoids (lycopene, alpha-carotene, and beta-carotene) were found in LDL, whereas the polar xanthophylls (capsanthin, lutein, and zeaxanthin) were distributed about equally between HDL and LDL (Oshima et al. 1997). The authors speculated that these polar xanthophylls might be localized at the polar surface of lipoproteins high in phospholipids (as is HDL) (Oshima et al. 1997).

Upon subfractionation of LDL particles, it was found that lycopene, beta-carotene and beta-cryptoxanthin are found mostly in larger, less-dense LDL particles whereas lutein and zeaxanthin are mostly in the smaller, more dense LDL particles (Lowe et al. 1999). Interestingly, the more dense LDL subfractions, which had lower overall carotenoid and vitamin E concentrations, were also more easily oxidized (Lowe et al. 1999).
Epidemiological and clinical data indicate that dietary antioxidants may protect against cardiovascular disease (Frei 1995). Several epidemiological studies have shown an inverse association between serum levels of beta-carotene and other carotenoids and coronary heart disease (reviewed by Kritchevsky 1999). One study found that serum levels of alpha- and beta-carotene and lycopene were 1.9-, 1.7-, and 2.7-fold higher, respectively, in Israeli men than in Czech men; mortality rates, blood pressure, and coronary heart disease rates in the subjects were highest in Czech and lowest in Israeli men (Bobak et al. 1999). However, clinical studies with carotenoid supplementation have been equivocal, and in fact some major clinical trials with beta-carotene supplementation have shown either no or negative effects on chronic diseases such as cardiovascular disease and cancer (reviewed by Mayne 1996 and Kritchevsky 1999). Carotenoids are regarded as good biomarkers for fruit and vegetable dietary intake, but other plant-derived compounds may well play a significant role in health. Still, studies have shown that supplementation with vitamin E (Reaven and Witztum 1993) and other small compounds (including vitamin C, beta-carotene and other carotenoids, and drugs such as probucol) can decrease the susceptibility of LDL to oxidation (Jialal and Fuller 1995); these compounds have in common their antioxidant activity.

Carotid intima-media thickness ("carotid IMT", essentially the thickness of one of the main arteries in the neck) is a measure of asymptomatic early atherosclerosis; in one atherosclerosis risk study, carotid IMT was found to be inversely correlated to the levels of lutein and zeaxanthin, which are xanthophylls (oxygenated carotenoids) regarded as biomarkers of fruit and vegetable intake (Irribaren et al. 1997). Another study found that lutein and cryptoxanthin were twice as high in a population (Toulouse) that had a much lower incidence of coronary heart disease than another group (Belfast), suggesting that such xanthophylls (hydroxycarotenoids) may be useful as antioxidant supplements (Howard et al. 1996).

Few studies have used carotenoids (other than beta-carotene) as anti-atherogenic dietary supplements. One *in vitro* study showed that cell-mediated oxidation of LDL was inhibited by beta-carotene, but enhanced by lutein or lycopene (Dugas et al. 1998). The same researchers later reported that dietary (i.e., *in vivo*) supplementation of 15 mg per day of beta-carotene over four weeks resulted in a 3- to 6-fold increase in the beta-carotene content of LDL; the *in vitro*-tested increase in oxidation resistance of LDL isolated from the subjects was greater than the increase in oxidation resistance seen in LDL enriched *in vitro* 11- to 12-fold with beta-carotene (Dugas 1999). Again, no effect on LDL resistance to oxidation was seen for lycopene supplied as a dietary supplement (Dugas 1999). These results are in contradiction to studies that reported a significant decrease in serum lipid peroxidation and LDL oxidation after three weeks of lycopene dietary supplementation (Agarwal and Rao 1998), and that *in vitro* supplementation of beta-carotene, canthaxanthin, or zeaxanthin inhibited cell-mediated LDL oxidation (Carpenter et al. 1997). A recent large study of the relationship between dietary antioxidant intake and risk for ischemic stroke (as a consequence of atherosclerosis) followed 43,738 men aged 40-75 years over 8 years (Ascherio et al. 1999). This study found a significant inverse relation between lutein intake and risk for ischemic stroke but this was not independent of other dietary factors. The authors concluded that vitamin E and vitamin C supplements and specific carotenoids did not substantially reduce risk for stroke in the population studied.
OXIDIERTES LDL

References:


Oxidiertes LDL


**Carotenoid Composition and Antioxidant Potential in Subfractions of Human Low-Density Lipoprotein.**

_Lowe GM, Bilton RF, Davies IG, Ford TC, Billington D, Young AJ._

School of Biomolecular Sciences, Liverpool John Moores University, UK. g.lowe@livjm.ac.uk

Carotenoids and vitamin E are transported in human plasma complexed with lipoproteins. The bulk of them are associated with low-density lipoprotein (LDL), in which form they may act as antioxidants and thus delay the onset of atherosclerosis. We used a simple, rapid, ultracentrifugation technique to fractionate plasma lipoproteins in self-generating gradients of iodixanol (Optiprep), a non-ionic iodinated density gradient medium. The carotenoid content and composition of a number of LDL subfractions was determined by reversed-phase high-performance liquid chromatography. Lycopene, beta-carotene and beta-cryptoxanthin were mainly located in the larger, less-dense LDL particles whereas lutein and zeaxanthin were found preferentially in the smaller, more dense LDL particles. When the antioxidant content of these fractions was expressed per milligram of LDL protein, significantly lower concentrations of carotenoid and vitamin E were found to be associated with the smaller, protein-rich fractions of LDL. Strong positive correlations were found between total carotenoid and vitamin E plasma concentrations and the lag-time of Cu(2+)-mediated oxidation of LDL subfractions. The more dense LDL subfractions, which had lower levels of these antioxidants, were more readily oxidized, highlighting their possible role in atherosclerotic events.
Oxidized LDL Associated With Metabolic Syndrome

Authors and Disclosures

Sue Hughes
Disclosure: Sue Hughes has disclosed no relevant financial relationships.

Charles Vega, MD
Disclosure: Charles Vega, MD, has disclosed an advisor/consultant relationship to Novartis, Inc.

Brande Nicole Martin
Disclosure: Brande Nicole Martin has disclosed no relevant financial information.

May 21, 2008 — A higher concentration of oxidized low-density lipoprotein (LDL)-cholesterol was associated with increased incidence of metabolic syndrome overall, as well as its components of abdominal obesity, hyperglycemia, and hypertriglyceridemia in a new population-based study [1].

The study, published in the May 21, 2008 issue of the Journal of the American Medical Association, was conducted by a team led by Dr Paul Holvoet (Katholieke Universiteit Leuven, Belgium).

Senior author Dr David Jacobs (University of Minnesota, Minneapolis) commented to heartwire: "This is another piece of evidence suggesting that oxidized LDL is harmful and is a signal of future heart disease risk even in people who are currently young and healthy."

The authors explain that studies in cellular and animal models have suggested that oxidized LDL, which accounts for only a minor fraction of LDL (0.001%-5%), contributes to processes that lead to the incidence of the metabolic syndrome, but this association has not been tested in humans.

They therefore set out to determine the association between the concentration of oxidized LDL and the incidence five years later of metabolic syndrome and its components in the population-based Coronary Artery Risk Development in Young Adults (CARDIA) study.

The CARDIA study followed 5115 participants who were between the ages of 18 and 30 years at the time of recruitment in 1985-1986 from four US metropolitan areas for 20 years. Oxidized LDL was assessed in 2823 participants at year 15 (2000-2001) as part of the Young Adult Longitudinal Trends in Antioxidants (YALTA) ancillary study. After the exclusion of participants who were pregnant, did not fast for at least eight hours before the test, had missing data, or who already had metabolic syndrome, the remaining 1889 patients were included in this study.
OXIDIERTES LDL

Results from the 20-year examination showed that 243 (12.9%) of these 1889 participants had developed metabolic syndrome. After adjustment for many different variables, oxidized LDL showed a graded relation to incident metabolic syndrome, with those in the highest quintile of oxidized LDL having a 3.5-times increased risk of metabolic syndrome compared with those in the lowest.

**Adjusted odds ratios (ORs) for incident metabolic syndrome after five-year follow-up by quintiles of oxidized LDL**

<table>
<thead>
<tr>
<th>Quintile of oxidized LDL</th>
<th>OR (95% confidence interval [CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (&lt;55.4 U/L)</td>
<td>1</td>
</tr>
<tr>
<td>2 (55.4 - 69.1 U/L)</td>
<td>2.1 (1.1 - 3.8)</td>
</tr>
<tr>
<td>3 (69.2 - 81.2 U/L)</td>
<td>2.4 (1.3 - 4.3)</td>
</tr>
<tr>
<td>4 (81.3 - 97.3 U/L)</td>
<td>2.8 (1.5 - 5.1)</td>
</tr>
<tr>
<td>5 (&gt;97.4 U/L)</td>
<td>3.5 (1.9 - 6.6)</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, race, study center, cigarette smoking, body-mass index, physical activity, and LDL-cholesterol levels

High levels of oxidized LDL were also associated with three of the individual components of metabolic syndrome—obesity, hypertriglyceridemia, and high fasting glucose—but were not associated with raised blood pressure or high-density lipoprotein (HDL) cholesterol.

**Adjusted odds ratios for incidence of metabolic-syndrome components in the highest vs. the lowest quintile of oxidized LDL**

<table>
<thead>
<tr>
<th>Metabolic-syndrome component</th>
<th>OR highest vs lowest quintile (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal obesity</td>
<td>2.1 (1.2 - 3.6)</td>
</tr>
<tr>
<td>High fasting glucose</td>
<td>2.4 (1.5 - 3.8)</td>
</tr>
<tr>
<td>High triglycerides</td>
<td>2.1 (1.1 - 4.0)</td>
</tr>
</tbody>
</table>

Adjusted for age, sex, race, study center, cigarette smoking, body-mass index, physical activity, and LDL-cholesterol levels
Oxidiertes LDL

These associations remained significant after adjustment for C-reactive protein, adiponectin, and antihypertensive, antidiabetic, and cholesterol-lowering medication.

In contrast, LDL cholesterol showed only a limited relation with metabolic syndrome, which the authors note tended to become flat in the fully adjusted model including oxidized LDL.

They conclude: "As yet, it is not possible to conclude whether oxidized LDL is a marker related to mechanistic underlying factors on the pathway to the development of metabolic syndrome or whether it is by itself a functional intermediary in this pathway. However, the strong association of oxidized LDL with the incidence of metabolic syndrome is consistent with a causal role."

Source

The complete contents of Heartwire, a professional news service of WebMD, can be found at www.theheart.org, a Web site for cardiovascular healthcare professionals.

Clinical Context

Although LDL does not become completely oxidized in the bloodstream, the fraction of partially oxidized LDL can range from as little as 0.001% among healthy adults to 5% among patients with acute coronary events. Small, dense LDL is particularly prone to oxidation.

Patients with the metabolic syndrome have been demonstrated to have higher levels of oxidized LDL compared with control patients, but there has been no research regarding whether higher concentrations of oxidized LDL can predict the development of the metabolic syndrome. The current study addresses this issue.

Study Highlights

- Participants in the Cardiovascular Risk Development in Young Adults Study were between the ages of 18 and 30 years at the inception of the research. Study recruitment occurred in 1985 and 1986.
- Oxidized LDL was assessed in 2823 subjects in year 15 of the study. Pregnant women were excluded from this analysis, as were participants with the metabolic syndrome at year 15.
- The metabolic syndrome was defined by the presence of at least 3 of the following 5 criteria, following a standard definition:
  - Waist circumference of at least 102 cm in men and 88 cm in women
  - Fasting triglycerides of 150 mg/dL or more
  - HDL cholesterol less than 40 mg/dL in men or less than 50 mg/dL among women
  - Blood pressure of at least 130/85 mm Hg or the use of antihypertensive medications
  - Fasting glucose of at least 100 mg/dL or the use of antidiabetic medication
The main study outcome was the relationship between oxidized LDL and the incidence of the metabolic syndrome at year 20 of the study. This result was adjusted to account for age, sex, race, cigarette smoking, body mass index, physical activity, and total LDL level.

Oxidized LDL was positively associated with male sex, black race, body mass index, and serum levels of C-reactive protein. However, total LDL cholesterol was unrelated to race or levels of C-reactive protein.

12.9% of the study cohort developed the metabolic syndrome by study year 20.

Compared with the lowest quintile of oxidized LDL, the ORs for developing the metabolic syndrome were as follows:

- Second quintile: 2.1
- Third quintile: 2.4
- Fourth quintile: 2.8
- Fifth quintile: 3.5

Total LDL cholesterol was not significantly associated with the risk for incident metabolic syndrome on fully adjusted analyses.

Comparing the highest vs lowest quintile of oxidized LDL on the OR of individual components of the metabolic syndrome, high levels of oxidized LDL significantly increased the risk for high fasting glucose (OR, 2.4), abdominal obesity (OR, 2.1), and elevated triglycerides (OR, 2.1). However, oxidized LDL was not a significant risk factor for blood pressure or HDL cholesterol.

**Pearls for practice**

- Oxidized LDL is only a small fraction of the total number of LDL particles in adults but is more prevalent among patients with coronary heart disease and the metabolic syndrome. Small, dense LDL is particularly prone to oxidation.
- The current study demonstrates that oxidized LDL is positively associated with the risk of developing the metabolic syndrome, particularly with regard to the metabolic syndrome components of abdominal obesity, hyperglycemia, and hypertriglyceridemia.
Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women\textsuperscript{1,2,3,4,5}

Tanja Weinbrenner, Helmut Schröder, Veronica Escurriol, Montserrat Fito, Roberto Elorza, Joan Vila, Jaume Marrugat and Maria-Isabel Covas

\textsuperscript{1} From the Department of Public Health, Universidad Miguel Hernández, Campus San Juan, Alicante, Spain (TW) and the Lipids and Cardiovascular Epidemiology Research Unit, Institut Municipal d'Investigació Mèdica (HS, VE, MF, RE, JV, JM, and M-IC), Barcelona, Spain

Background: Obesity is associated with oxidative stress, and the oxidation of LDL is thought to play a crucial role in the generation of atherosclerotic lesions.

Objective: The objective was to describe the association of waist circumference (WC) and body mass index (BMI; in kg/m\textsuperscript{2}) with plasma circulating oxidized LDL (ox-LDL) and C-reactive protein (CRP).

Design: This cross-sectional study included data for circulating ox-LDL and CRP from a subpopulation of 586 men and women enrolled in a population-based survey conducted in 2000 in Girona, Spain. Multivariate analysis was performed to describe the independent association of WC and BMI with ox-LDL and CRP.

Results: Multivariate logistic regression analysis adjusted for lifestyle, educational level, and dietary confounders showed a direct association of WC (quartile distribution) and BMI categories with ox-LDL (P for linear trend = 0.002) and CRP (P for linear trend = 0.004). Subjects in the top quartile of WC and with a BMI > 29.9 were at high risk of elevated circulating concentrations of ox-LDL and CRP. Further adjustment for cardiovascular disease risk factors did not substantially modify these associations. The risk of high ox-LDL concentrations in overweight (BMI = 25.0–29.9) or obese (BMI \geq 30) subjects with a WC < 102 cm (men) or < 88 cm (women) was not significantly different from that in normal-weight subjects with these WCs. In contrast, overweight or obese subjects with higher WCs (WC \geq 102 cm for men and \geq 88 cm for women) were at significantly higher risk of increased ox-LDL.

Conclusion: High WC was associated with high concentrations of ox-LDL independently of BMI in the study population.

Key Words: Oxidized LDL • abdominal obesity • cardiovascular disease risk factors • weight • BMI
PPARgamma agonists enhance human vascular endothelial adhesiveness by increasing ICAM-1 expression.

Chen NG, Sarabia SF, Malloy PJ, Zhao XY, Feldman D, Reaven GM.

Department of Medicine, Stanford University School of Medicine, Stanford, California 94305, USA.
jchen@gcrc.stanford.edu

Early atherosclerotic lesions are characterized by increased monocyte adhesion to the overlying endothelium. Oxidized LDL (oxLDL) stimulates the adhesion of human monocytes to endothelial cells, in part, by increasing expression of ICAM-1. However, the cellular role of oxLDL in endothelial adhesiveness is not well understood. The peroxisome proliferator-activated receptor gamma (PPARgamma), a member of the nuclear receptor superfamily, is expressed in vascular endothelial cells. Whether it can be activated by a synthetic ligand, troglitazone, as well as by natural ligands, oxLDL and its lipid components (i.e., 9- and 13-HODE), has not yet been explored. This study was undertaken to determine whether PPARgamma is expressed in ECV304 human vascular endothelial cells and if so to define the biological effects of its activation by these agonists. Our results demonstrate that PPARgamma mRNA is expressed in ECV304 cells, and transfected cells with a PPARE luciferase construct respond to these agonists. In addition, ligand-dependent PPARgamma activation increased ICAM-1 protein expression and enhanced adherence of monocytes to ECV304 cells by two- to threefold. These findings suggest that the PPARgamma signaling pathway might contribute to the atherogenicity of oxLDL in vascular endothelial cells. Copyright 1999 Academic Press.