AFGIFTEKANTOOR LEUVEN 1
P5 08820
Postbus 1212, 3000 Leuven 1

# Lipid Club Letter

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**Contents**





## *Editorial*

*Dear Colleagues,*

*In this letter of the Belgian Lipid Club, You will find contributions from different authors on a variety of issues that relate to atherogenesis and its clinical consequences.*

*Science is advancing very fast; it is not easy to capture all the new information that becomes available; I thank all the authors for helping us on this by submitting interesting manuscripts for the Lipid Club letter.*

*May I remind You all of the Atherothrombosis summit that is co-organised by the Belgian Lipid Club in Leuven on december 18th 2009.*

*We were able to bring together high-ranking scientists to discuss various issues related to atherosclerosis and thrombosis. I hope that You will make use of this opportunity to meet and to discuss with top experts in the field of atherothrombosis.*

*I wish You interesting reading of this Letter and I hope to see You at our next meeting.*

*Cordially*

*Prof. Dr. G. De Backer*
*President BLC*



Responsible editor: Prof. G. DE BACKER (R.U. Gent) Editorial staff: Prof. J. DUCOBU (ULB, UMH)

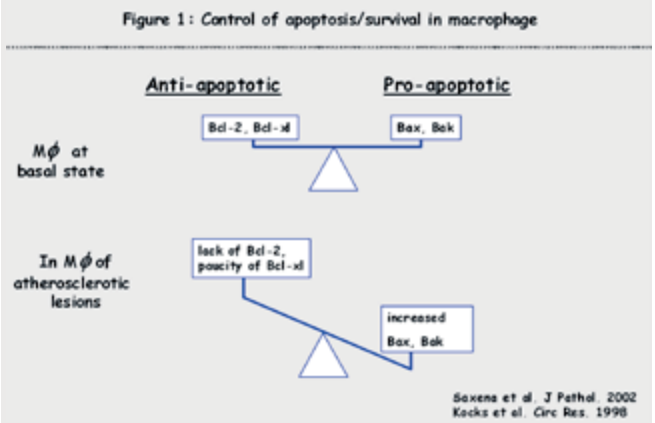
# ROLE OF MACROPHAGE SURVIVAL/APOPTOSIS ON ATHEROGENESIS.

**Philippe Lesnik and Emmanuel Gautier**

Unité Mixte de Recherche INSERM U-939 / Université Pierre et Marie Curie, Paris

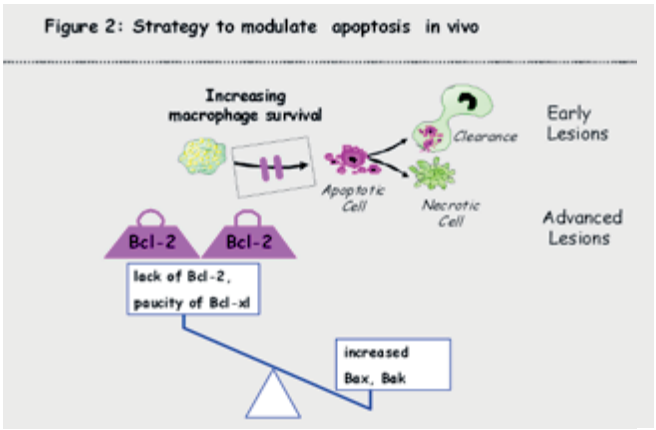
Atherosclerosis is an inflammatory vascular disease characterized by the intimal accumulation of macrophage foam cells, cell death and chronic arterial inflammation [1-3]. Macrophage apoptosis has been identified as a prominent feature of atherosclerotic plaques and, as macrophages represent the main cell type that undergoes apoptosis in the lesion, macrophage cell death is believed to support necrotic core growth. However, the impact of macrophage apoptosis on plaque progression still has to be specifically investigated.

Apoptotic process is controlled by intracellular levels of pro- and anti-apoptotic proteins such as those of the Bcl-2 family. Indeed, the relative expression of pro- (e.g. Bax and Bak) and anti-apoptotic proteins (e.g. Bcl-2 and Bcl-xL) of the Bcl-2 family determines the overall sensitivity of the cell to apoptotic stimuli [4]. In macrophages of atherosclerotic lesions, the pro-apoptotic Bax and Bak proteins predominate, while anti-apoptotic Bcl-2 and Bcl-xL are deficient [5-7], thereby arguing for an enhanced susceptibility to apoptosis (figure 1).

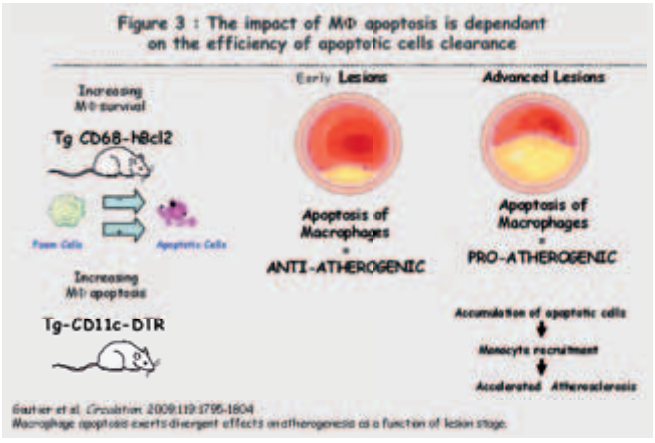



Recent studies have shed light on the potential impact of apoptosis on atherosclerotic lesion progression. Indeed, the disruption of either the pro-apoptotic molecule Bax in bone marrow-derived cells [8], or the anti-apoptotic factor AIM [9], has revealed that apoptosis attenuates early plaque formation, while the depletion of Bcl-2 in macrophages had no effect on plaque progression [10]. However, as apoptotic cells accumulate preferentially in advanced rather than in early lesions [11, 12], macrophage apoptosis may differentially influence plaque progression as a function of lesion stage [13]. In fact, apoptotic cell clearance appears to be defective in advanced lesions, but in contrast, efficient in early ones [14]. Moreover, apoptotic cells may have pro-inflammatory properties, in part due to the presence of oxidised phospholipids at their surface [15, 16], which are known triggers of inflammatory responses in arterial tissues [17]. In this setting, studies of mice in which components of the apoptotic cell clearance machinery such as Mfg-e8, TG2 C1q and MerTK Receptor [18-20] have been deleted, or of lupus-prone mice characterized by ineffective apoptotic cell clearance [21, 22], have indeed revealed that defective apoptotic cell clearance is associated with enhanced atherosclerotic plaque progression.

Consequently, macrophage apoptosis may differentially impact plaque progression as a function of lesion stage. Nevertheless, this putative scenario has not been substantiated by direct in vivo experimental data. To address this critical question, we developed an experimental strategy based on the specific modulation of macrophage survival in atherosclerotic lesions in vivo. We first used a transgenic approach allowing specific protection of macrophages against apoptosis (CD68-hBcl-2 mice). We demonstrated that macrophage survival exerts pro-atherogenic effects during the early stages of atherosclerotic plaque progression, while it reduced plaque burden when lesions were at a more advanced stage (figure 2). To mirror




this effect, we applied a complementary approach based on chronic induction of lesional macrophage apoptosis in CD11c-DTR transgenic mice, and demonstrated that apoptotic cell accumulation in advanced lesion enhances plaque progression. Finally, acute induction of macrophage apoptosis provided evidence that apoptotic cell accumulation in advanced atherosclerotic lesions promotes inflammatory gene expression, circulating monocyte recruitment and accumulation of newly-recruited macrophages [23] (figure 3).



In summary, the present study established that there is a divergent impact of apoptosis on plaque progression as a function of the stage of lesion development. In early lesions, apoptotic death of lesional macrophages is atheroprotective, whereas in advanced lesions, macrophage apoptosis triggers pro-inflammatory signals facilitating the recruitment of monocytes and subsequent lesion growth.

Therefore, attenuated macrophage apoptosis may provide a new potential therapeutic strategy to delay long-term plaque progression and future investigations should address whether this inhibition of macrophage death from early stages of the disease could be beneficial in terms of overall lesion progression.

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# OXLDL AND PRECLINICAL ATHEROSCLEROSIS: LESSONS FROM THE ASKLEPIOS STUDY.


**Michel Langlois,[1,2] Ernst Rietzschel,[2] Marc De Buyzere[2]**

[1] Dept. of Clinical Chemistry, AZ St-Jan Brugge
[2] Dept. of Cardiovascular Diseases, Ghent University


Low-density lipoproteins (LDL) are susceptible to structural modifications by oxidation, particularly the small dense LDL particles. Oxidized LDL (oxLDL) formation in the subendothelial space of the arterial wall is a key initiating step in atherosclerosis and it contributes to endothelial dysfunction and inflammatory processes. Progress in the development of immunoassays, using monoclonal antibodies against oxidation-dependent epitopes of LDL, made it possible to directly measure oxLDL in the circulation. Increased circulating oxLDL concentrations have been related to coronary heart disease in some studies, and also reflect metabolic disorders including atherogenic dyslipidemia and the metabolic syndrome. The ASKLEPIOS study in 2524 apparently healthy subjects, aged 35-55 years (51% females), free from overt cardiovascular disease, investigated the interaction between in-vitro biomarkers, lifestyle and vascular imaging (ultrasound examination of carotid and femoral arteries). We found that plasma oxLDL (mAb-4E6) is elevated in subjects with preclinical atherosclerosis, assessed by measuring intima-media thickness (IMT) and plaque. Circulating OxLDL was independently associated with femoral plaque, but not with carotid atherosclerosis [(1)]. This can be explained by the fact that femoral arteries are more susceptible to oxidative stress conditions, smoking and diabetes, whereas hypertension is a major factor contributing to carotid vascular alterations. Furthermore, subjects with femoral atherosclerosis show a higher plaque burden. Assuming that atheroma in the vascular wall is a major site of production of oxLDL, that diffuses into the circulation, leakage of oxLDL from atheroma in subjects with (preclinical) femoral arterial disease makes a significant proportionate contribution to total circulating oxLDL. As such, oxLDL could be a promising early marker of clinically silent, atherosclerotic plaque. However, the clinical significance of measuring oxLDL for cardiovascular risk assessment or prevention strategies remains unclear.

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# LIFE EXPECTANCY IN RELATION TO CARDIOVASCULAR RISK FACTORS: 38 YEAR FOLLOW-UP OF 19,000 MEN IN THE WHITEHALL STUDY.

**J. DUCOBU (Université de Mons) from the following BMJ paper :**
**BMJ. 2009 Sep 16; Clarke R, Emberson J, Fletcher A, Breeze E, Marmot M, Shipley MJ.**

Clinical Trial Service Unit and Epidemiological Studies Unit (CTSU), Richard Doll Building, University of Oxford

Rather than explaining to patients the reduction of risk induced by changes in lifestyle or by compliance to drugs, it is probably more relevant to insist on the increase in lifetime related to theses changes.
This very important study assesses life expectancy in relation to cardiovascular risk factors.
The Whitehall study is a prospective cohort study which includes18,863 men employed in the civil service in London, England. The participants were examined at entry in 1967-70 and followed for 38 years, of whom 13,501 died and 4811 were re-examined in 1997.
The life expectancy was estimated in relation to fifths and dichotomous categories of risk factors (smoking, "low" or "high" blood pressure (>or=140 mm Hg), and "low" or "high" cholesterol (>or=5 mmol/l)), and a risk score from these risk factors.

At entry, 42% of the men were smokers, 39% had high blood pressure, and 51% had high cholesterol. Upon re-examination, about two thirds of the previous smokers had quit smoking shortly after entry and the mean differences in levels of those with high and low levels of blood pressure and cholesterol were attenuated by two thirds. Compared with men without any baseline risk factors, the presence of all three risk factors at entry was associated with **a 10 year shorter life expectancy from age 50 (23.7 v 33.3 years)**. Compared with men in the lowest 5% of a risk score based on smoking, diabetes, employment grade, and continuous levels of blood pressure, cholesterol concentration, and body mass index (BMI), men in the highest 5% had **a 15 year shorter life expectancy from age 50 (20.2 v 35.4 years**).

| Risk score based on BP, cholesterol, smoking, BMI, grade, glucose intolerance and diabetes | Vascular deaths OR | Non-vascular deaths OR | Life expectancy at age 50 (SE) |
|---|---|---|---|
| **Lowest 5 %** | 1.0 | 1.0 | 35.4 (0.4) |
| **Middle 90 %** | 18 (1.86-2.56) | 1.68 (1.47-1.92) | 29.3 (0.1) |
| **Highest 5 %** | 4.65 (3.86-5.60) | 2.7 (2.28-3,2) | 20.2(O.6) |

Despite substantial changes in these risk factors over time, baseline differences in risk factors were associated with 10 to 15 year shorter life expectancy from age 50.
The results provide support for the public health policies aimed at achieving modest changes in major risk factors throughout the population to achieve improvements in life expectancy.

Continued public health strategies to lower mean levels of the three main cardiovascular risk factors, together with more intensive medical treatment for "high risk" subgroups ,including use of medication to lower blood pressure and cholesterol concentration, that have proven efficacy could result in further improvements in life expectancy.

### What is already known on this topic

- **There has been uncertainty about the limits of life expectancy and the relevance of cardiovascular risk factors for prediction of life expectancy**

### What this study adds

- **Despite substantial variability within individuals in levels of cardiovascular risk factors, the presence of three major risk factors (smoking, high blood pressure, and high cholesterol concentration) recorded on a single occasion in middle-aged men was associated with a 10 year shorter life span from age 50 (23.7 v 33.3 years) compared to men with none of these risk factors.**
- **More extreme categorisation of these risk factors including BMI, diabetes mellitus/glucose intolerance, and employment grade was associated with a 15 year difference in life expectancy from age 50 (20.2 v 35.4 years) Continued public health strategies to lower these risk factors could result in further improvements in life expectancy**

# A NEW DEFINITION OF THE METABOLIC SYNDROME BY IDF, NHLBI, WHF, IAS AND AHA

**J. DUCOBU**

Université de Mons

A new joint statement from a number of professional organizations (the **International Diabetes Federation** (IDF), the **National Heart**, **Lung**, and **Blood Institute** (NHLBI), the **World Heart Federation**, the **International Atherosclerosis Society**, and the **American Heart Association** (AHA))has identified specific criteria for the clinical diagnosis of the metabolic syndrome, tightening up the definition, which previously differed from one organization to the next.
The statement, published online October 5, 2009 in Circulation, is an attempt to eliminate some of the confusion surrounding the identification of patients with the syndrome.
The **IDF** definition and the **National Cholesterol Education Program Adult Treatment Panel** ATP III definition are the two that have been used most frequently.
Specifically, the new metabolic syndrome definition streamlines previous differences related to abdominal obesity as defined by measurements in waist circumference. Substantial disparities existed between the previous IDF and the ATP III definitions of what constituted an excessively large waist circumference, by as much as 8 cm between the two groups.
Now, the criteria for elevated waist circumference are based on population- and country-specific definitions. The problem that still exists is that regional differences around the world may be substantial in terms of what waist circumference infers as regards additional risk for heart disease and diabetes.

| | | Recommended Waist Circumference Threshold for Abdominal Obesity | |
|---|---|---|---|
| Population | Organization (Reference) | Men | Women |
| Europid | IDF (4) | ≥94 cm | ≥80 cm |
| Caucasian | WHO (7) | ≥94 cm (increased risk)<br>≥102 cm (still higher risk) | ≥80 cm (increased risk)<br>≥88 cm (still higher risk) |
| United States | AHA/NHLBI (ATP III)* (5) | ≥102 cm | ≥88 cm |
| Canada | Health Canada (8,9) | ≥102 cm | ≥88 cm |
| European | European Cardiovascular Societies (10) | ≥102 cm | ≥88 cm |
| Asian (including Japanese) | IDF (4) | ≥90 cm | ≥80 cm |
| Asian | WHO (11) | ≥90 cm | ≥80 cm |
| Japanese | Japanese Obesity Society (12) | ≥85 cm | ≥90 cm |
| China | Cooperative Task Force (13) | ≥85 cm | ≥80 cm |
| Middle East, Mediterranean | IDF (4) | ≥94 cm | ≥80 cm |
| Sub-Saharan African | IDF (4) | ≥94 cm | ≥80 cm |
| Ethnic Central and South American | IDF (4) | ≥90 cm | ≥80 cm |

*Recent AHA/NHLBI guidelines for metabolic syndrome recognize an increased risk for CVD and diabetes at waist-circumference thresholds of ≥94 cm in men and ≥80 cm in women and identify these as optional cut points for individuals or populations with increased insulin resistance.

The IDF previously considered elevations in waist circumference as mandatory when defining metabolic syndrome, although the ATP III did not.
Now, **waist circumference is just one of five criteria** that physicians can use when diagnosing the metabolic syndrome.

**Patients with three of the five criteria, i.e. elevated waist circumference, elevated triglycerides, reduced HDL-cholesterol levels, are considered to have the syndrome**.

| Measure | Categorical cut-off points |
|---|---|
| **Elevated waist circumference** | Population- and country-specific definitions |
| **Elevated triglycerides (or drug treatment for elevated triglycerides)** | >150 mg/dl |
| **Reduced HDL cholesterol (or drug treatment for reduced HDL cholesterol )** | <40 mg/dL for males and <50 mg/dl for females |
| **Elevated blood pressure (or drug treatment for elevated blood pressure)** | Systolic >130 mm Hg and/or diastolic >85 mm Hg |
| **Elevated fasting glucose (or drug treatment for elevated glucose)** | >100 mg/dl |

Absent from the joint statement is the **American Diabetes Association**. Specifically, the **ADA**, as well as the **European Association for the Study of Diabetes (EASD)**, objected to the manner in which the metabolic syndrome was characterized as a risk factor for heart disease or diabetes, arguing that there was no need to diagnose a patient with the syndrome because emphasis should be placed on aggressively treating the individual risk factors. In 2005, the ADA and EASD issued their own joint statement calling for a critical appraisal of metabolic syndrome, its designation as a syndrome, and its clinical utility.

Metabolic syndrome is not a disease but a clustering of risk factors. The goal of identifying the syndrome was to draw clinicians' and the public's attention to the importance of a high-quality lifestyle, and metabolic syndrome should not be used as a predictor of heart disease or diabetes risk.

It should also help to clarify the comparisons between clinical studies on metabolic syndrome and stimulate more basic research on this very important topic

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Alberti KG, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome. A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 2009; 120:1640-1645

# CARDIOVASCULAR DISEASES, ERECTILE DYSFUNCTION AND MYELOPEROXIDASE

**Thierry Roumeguère[1,2], Pierre van Antwerpen[3], Karim Zouaoui Boudjeltia[1]**

[1] Laboratoire de Médecine Expérimentale, unité 222, Université Libre de Bruxelles, CHU Charleroi, site André Vésale, Montigny-le-Tilleul
[2]Service d'Urologie, Hôpital Erasme, Brussels
[3] Laboratoire de Pharmacologie, Institut de Pharmacie, Université Libre de Bruxelles

*[Already published in Andrologic 2009; 5(4): 108-110]*

***Erectile dysfunction*** (ED) and ***cardiovascular diseases*** have numerous risk factors in common, such as diabetes, lipid disorders, obesity and the metabolic syndrome [Roumeguère Th, Wespes E (2007) Troubles érectiles et maladies cardiovasculaires en pratique clinique. Rev Med Brux 28: 360-6]. Associated with multiple chronic pathologies and health-risk factors, such as smoking and sedentary lifestyle, erectile dysfunction cannot be seen as exclusively a sexual disorder. The growing interest in erectile disorders resides in the fact that they can now be considered as ***early markers*** for coronary disease and provide grounds for screening for an asymptomatic cardiovascular pathology *[Vlachopoulos C, Rokkas K, Ioakeimidis N, et al. (2005)Prevalence of asymptomatic coronary artery disease in men with vasculogenic erectile dysfunction: a prospective angiographic study. Eur Urol;48:996–1003.; Montorsi P., Montorsi F., Schulman C. Is erectile dysfunction the "tip of the iceberg" of a systemic vascular disorder? Eur.Urol. 2003; 44: 352-54].*

Several studies have confirmed the relationship between dyslipidaemia and erectile dysfunction *[Roumeguère Th, Wespes E, Carpentier Y, et al. (2003) Erectile dysfunction is associated with a high prevalence of hyperlipidemia and coronary heart disease risk. Eur Urol 44: 355-9, Ponholzer A, Temml C, Obermayr R, et al. (2005) Is Erectile Dysfunction an Indicator for Increased Risk of Coronary Heart Disease and Stroke? Eur Urol 48: 512-8].* It would appear that the severity of erectile dysfunction is related to the level of hyperlipidaemia *[Shabsigh R, Perelman M, Lockhart D, et al. (2005) Health issues of men: prevalence and correlates of erectile dysfunction. J Urol 174: 662-667].*

***Obesity*** is also directly linked to the presence of an erectile disorder with a relative risk of 2, once the body-mass index is greater than 30 kg/m2 *[Ponholzer A, Temml C, Obermayr R, et al. (2005) Is Erectile Dysfunction an Indicator for Increased Risk of Coronary Heart Disease and Stroke? Eur Urol 48: 512-8].* It is important to remember that it is essentially the increase in visceral fat which must be considered. This risk factor increases with the number of modifiable components of the metabolic syndrome *[Esposito K, Giugliano F, Martedì E, et al. (2005) High Proportions of Erectile Dysfunction in Men With the Metabolic Syndrome Diabetes Care 28: 1201-3].*

The ***metabolic syndrome*** has an appreciable impact in the pathogenesis of erectile disorders, at least in men over 45 years of age, where pathology of the ***endothelium*** represents the link between the vascular risk factors of this syndrome. *[Heidler S, Temml C, Broessner C, et al. (2007) Is the Metabolic Syndrome an Independent Risk Factor for Erectile Dysfunction? J Urol 177: 651-54].* Endothelial damage is accompanied by an increase in the principal inflammation markers, such as CRP and the interleukins IL6 and IL8 *[Esposito K, Giugliano F, Di Palo C, et al. (2004) Effect of lifestyle changes on erectile dysfunction in obese men: a randomized controlled trial. JAMA 291: 2978].* All the risk factors responsible for oxidative stress are involved in the endothelial damage responsible for the development of erectile dysfunction and cardiovascular diseases *[Billups K (2005) Erectile dysfunction as a marker for vascular disease. Curr Urol Rep 6: 439-44].*

## Erection, Endothelium and Atherosclerosis

Normal erectile function depends on the balance between the contraction and relaxation of intrapenile smooth muscle fibres. An erection requires the dilation of the arteries of the penis, a significant increase in blood flow and engorgement of the corpus cavernosum. The slightest haemodynamic anomaly can be sufficient to bring about erectile disorders. The principal erection neurotransmitter is ***nitric oxide (NO)***. The 2 main sources of NO in the penis are the parasympathetic nerve fibres and the endothelial cells present in the blood vessels of the cavernous sinusoids. The synthesis of NO is influenced by the state of the endothelium and the presence of oxygen. NO activates guanylate cyclase (GC) which is responsible for the conversion of guanosine tri-phosphate (GTP) to cyclic guanosine mono-phosphate (cGMP). The accumulation of cGMP activates a G protein kinase (GPK) which causes a drop in intracellular Ca2+,resulting in the relaxation of the smooth muscle responsible for the erection. An anomaly of the NO / cGMP system can lead to the development of an erectile disorder which may be the first sign of cardiovascular disease. *[Piero Montorsi, Thierry Roumeguère, Francesco Montorsi, Paolo M. Ravagnani, Stefano Galli, Alberto Briganti, Andrea Salonia, Claude C. Schulman (2004) Is There a Link between Erectile Dysfunction and Coronary Artery Disease? EAU Update Series 2 43–48].* Atherosclerosis causes a reduction in oxygen and NO levels and this leads to fibrosis and atrophy of the corpus cavernosum. The link between hyperlipidaemia and erectile dysfunction is atherosclerosis, which affects the relaxation of the smooth muscle of the corpus cavernosum through impairment of endothelial function. *[Azadzoi K., Saenz de Tejada I. (1991) Hypercholesterolemia impairs endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle. J.Urol.; 146: 238.].*

The physiopathological mechanism common to erectile disorders and cardiovascular diseases would therefore seem to be an ***impairment of endothelial function*** which plays a major role both in the relaxation of smooth muscle mediated by NO and in regulation of the circulation. The impairment of endothelial function precedes the formation of atheromatous plaque *[Solomon H., Man JW., Jackson G. Erectile dysfunction and the cardiovascular patient: Endothelial dysfunction is the common denominator. Heart. 2003; 89: 251–3 ; Kirby M, Jackson G, Simonsen U. (2005)Endothelial dysfunction links erectile dysfunction to heart disease. Int J Clin Pract;59:225–9.].*

The symptoms and development over time of atherosclerosis can be variable, depending on their magnitude and location, there being variations in the size of the arteries according to the territories supplied (penis, heart, brain, lower limbs). Therefore, even tiny lesions can impair penile circulation without there being any affect on carotid or coronary flow. At a more advanced stage, obstruction of 50% of the lumen of the coronary arteries will cause a specific symptomatology (angina etc.), with an even greater obstruction of penile circulation *[Montorsi P, Ravagnani PM, Galli S, Rotatori F, Briganti A, Salonia A, Rigatti P, Montorsi F. The artery size hypothesis: a macrovascular link between erectile dysfunction and coronary artery disease. Am J Cardiol. 2005 Dec 26;96(12B):19M-23M].*

In the initial stage of atherosclerosis screening tests for coronary disease can prove normal in almost fifty percent of cases. However, if atherosclerosis is not recognised at an early stage and becomes extensive, erectile dysfunction becomes just one of the vascular symptoms resulting from it. *[Montorsi P, Ravagnani PM, Galli S, Rotatori F, Veglia F, Briganti A, Salonia A, Dehò F, Rigatti P, Montorsi F, Fiorentini C. Association between erectile dysfunction and coronary artery disease. Role of coronary clinical presentation and extent of coronary vessels involvement: the COBRA trial. Eur Heart J. 2006 Nov;27(22):2632-9]*

## Myeloperoxidase (MPO)

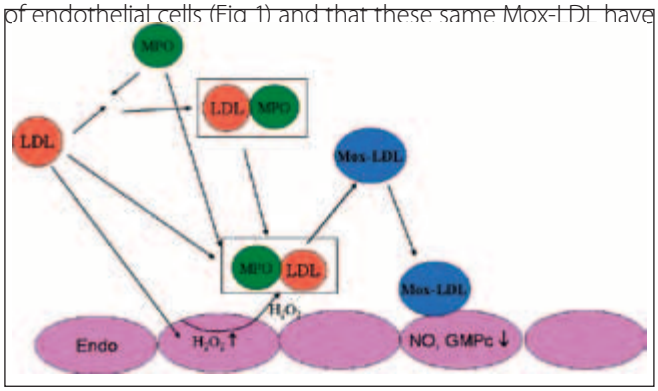
The oxidation theory suggests that oxidation of LDL is an early event in atherosclerosis and that the oxidised LDLs contribute to atherogenesis by triggering inflammation. Various lipoprotein oxidation routes have been described in vivo, among which ***myeloperoxidase (MPO)***.

MPO plays an important role in the atherosclerosis mechanism. It is partly responsible for the accumulation of oxidised LDL in the macrophages and for the development of atheromatous plaque. MPO levels are associated with endothelial dysfunction *[Vita JA, Brennan ML, Gokce N, Mann SA, Goormastic M, Shishehbor MH, Penn MS, Keaney JF, Jr., Hazen SL. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation. 2004; 110: 1134–1139].* Moreover, studies have shown that high plasma levels of MPO are predictors of cardiovascular events (infarction, coronary syndrome) *[Baldus, S., Heeschen, C., Meinertz, T., Zeiher, A.M., Eiserich, J.P., Munzel, T., Simoons, M.L., and Hamm, C.W. 2003. Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation, 108: 1440 1445. Brennan, M.L., Penn, M.S., Van Lente, F., Nambi, V., Shishehbor, M.H., Aviles, R.J., et al. 2003. Prognostic value of myeloperoxidase in patients with chest pain. N. Engl. J. Med. 349: 1595–1604.].*

MPO is a haem iron protein contained in the azurophilic granules of neutrophils; it catalyses the oxidation of chloride to hypochlorite (antiseptic agent) in the presence of hydrogen peroxide (H2O2). In the immune defence context, the MPO/H2O2/chloride system is under the strict control of the neutrophils and promotes the elimination of exogenic structures. However, in cases of oxidative stress the neutrophils release the content of their granules into the extracellular fluid. In such an event the body must deal with a so-called "circulating" MPO, which can cause significant tissue damage.

In order to define the physiopathological impact of MPO, a monoclonal antibody has been developed which specifically recognises the oxidation of low-density lipoproteins caused by the MPO/H2O2/Cl system (LDL). *[N. Moguilevsky, K. Zouaoui Boudjeltia, S. Babar, P. Delrée, I. Legssyer, Y. Carpentier, M. Vanhaeverbeek, J. Ducobu, Monoclonal antibodies against LDL progressively oxidized by myeloperoxidase react with ApoB-100 protein moiety and human atherosclerotic lesions, Biochem. Biophys. Res. Comm. 323 (2004)1223-1228.].*

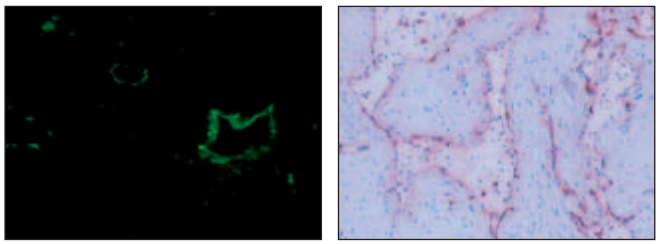
With the help of this original tool, it has been possible to show that LDLs can be oxidised by MPO (Mox-LDL) on the surface of endothelial cells (Fig 1) and that these same Mox-LDL have



a pro-inflammatory effect. The Mox-LDLs bring about an increase in the production of cytokines by the monocytes (TNF-alpha) and of IL-8 by the endothelial cells. These two independent effects can act in synergy to increase systemic or local inflammation and promote atherogenesis and the development of atherosclerosis. *[Zouaoui Boudjeltia, K.; Legssyer, I.; Van Antwerpen, P.; Kisoka, R.L.; Babar, S.; Moguilevsky, N.; Delree, P.; Ducobu, J.; Remacle, C.; Vanhaeverbeek, M.; Brohee, D. Biochem. Triggering of inflammatory response by myeloperoxidase-oxidized LDL. Biochem Cell Biol. 84 (2006) 805-812.]*. Furthermore, Mox-LDLs are responsible for the reduction in the synthesis of NO in the endothelial cells *[Nuszkowski A, Gräbner R, Marsche G, Unbehaun A, Malle E, Heller R. Hypochlorite-modified low density lipoprotein inhibits nitric oxide synthesis in endothelial cells via an intracellular dislocalization of endothelial nitric-oxide synthase J Biol Chem. 2001 Apr 27;276(17):14212-21112,]*

## MPO and Erectile Disorders

The presence of Mox-LDL has been reported in the corpus cavernosum in patients with erectile dysfunction of vascular origin (Fig2). *[Karim Zouaoui Boudjeltia , Thierry Roumeguere,*



*Paul Delree , Nicole Moguilevsky , Jean Ducobu , Michel Vanhaeverbeek , Eric Wespes ( 2 0 0 7) Presence of LDL Modified by Myeloperoxidase in the Penis in Patients with Vascular Erectile Dysfunction: A Preliminary Study European urology 51 262–269]*. In immunohistochemistry the Mox-LDLs are found on the surface and in the cytoplasm of endothelial cells, as well as here and there in smooth muscle cells (photo). MPO modulates the vascular inflammatory response by regulating the availability of NO. *[Vita JA, Brennan ML, Gokce N, Mann SA, Goormastic M, Shishehbor MH, Penn MS, Keaney JF, Jr., Hazen SL. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation. 2004; 110: 1134–1139]*. Disruption of the synthesis of NO by the Mox-LDLs provides a physiopathological hypothesis for endothelial-controlled erection disorders. This phenomenon has to be confirmed in the human through clinical studies.

The NO / cGMP system is dominant in the relaxation/contraction balance of the tonus of smooth muscles cells. We have recently shown the potential impact that Mox-LDLs could have on endothelium by reducing the levels of cGMP found in these cells. *[Roumeguère T, et al. (2000) Effect of LDL Modified by Myeloperoxidase-H2O2- Cl_ System on Intracellular Cyclic Guanosine Monophosphate Level of Endothelial Cells: A Link to Erectile Dysfunction? Eur Urol doi:10.1016/j.eururo. 2008.09.038]*. An increase in intracellular Ca2+ resulting from the absence of an accumulation of cGMP is responsible for a loss of relaxation in smooth muscle cells.

## Inhibitors of Type 5 Phosphodiesterase and Myeloperoxidase

The selective inhibitors of Type 5 phosphodiesterase (PDE5) are first-line treatment for erectile dysfunction; they oppose the regulatory action of phosphodiesterase 5 which hydrolyses cGMP. *[Wespes E, Amar E, Hatzichristou D, et al. EAU Guidelines on erectile dysfunction: an update. Eur Urol 2006;49:6–15]*. Non-selective inhibitors of phosphodiesterases participate in the reduction of inflammation (reduction of CRP and Interleukins (IL) 6 and 8) and promote an inflammatory response (TGF-β) in patients with atherosclerosis. *[Sliwa K, Woodiwiss A, Kone VN, et al. (2004). Therapy of ischemic cardiomyopathy with the immunomodulating agent pentoxifylline: results of a randomized study. Circulation;109:750–5]*.

NO exerts an anti-inflammatory action through the production of cGMP *[Ahluwalia A, Foster P, Scotland RS, et al.(2004) Anti-inflammatory activity of soluble guanylate cyclase: cGMP-dependent down-regulation of P-selectin expression and leukocyte recruitment. Proc Natl Acad Sci USA;101: 386–91]*, but paradoxically inflammation reduces the bioavailability of NO. A beneficial effect of PDE5 inhibitors in inflammation and in the improving of endothelial function has been described in several studies *[Aversa A, Vitale C, Volterrani M, et al. (2008). Chronic administration of sildenafil improves markers of endothelial function in men with type 2 diabetes. Diabet Med;25:37–44. ; Rosano GM, Aversa A, Vitale C, Fabbri A, Fini M, Spera G (2005). Chronic treatment with tadalafil improves endothelial function in men with increased cardiovascular risk. Eur Urol;47: 214–20 ; Mazo E, Gamidov S, Iremashvili V. (2006)The effect of vardenafil on endothelial function of brachial and cavernous arteries. Int J Impot Res;18:464–9.]* together with their potential long-term cardioprotective effect *[Bella A, De Young L, Mussa al-Numi, Brock G. Daily administration of phosphodiesterases type 5 inhibitors for urological and nonurological indications. Eur Urol 2007;52:990–1005.]*. As we already knew that Mox-LDLs increase the expression of vascular inflammation markers, such as Interleukin 8 (IL8) *[Zouaoui Boudjeltia K, Legysser I, Van Antwerpen P, et al. Triggering of inflammatory response by myeloperoxidase-oxidized LDL. Biochem Cell Biol 2006;84:805–12]*, we examined the effect of 3 PDE 5 inhibitors available for the treatment of erectile dysfunction - sildenafil, vardenafil and tadalafil – on the inflammatory response of endothelial cells stimulated by Mox-LDLs. ***In vitro***, the 3 PDE5 inhibitors by themselves had no influence on the production of IL8 by the endothelial cells. However, in the presence of Mox-LDL, tadalafil was the only PDE5 inhibitor to reduce the inflammatory response. *[Roumeguère T, Zouaoui Boudjeltia K, Babar S et al., (2009), Effects of Phosphodiesterase Inhibitors on the Inflammatory Response of Endothelial Cells Stimulated by Myeloperoxidase-Modified Low-Density Lipoprotein or Tumor Necrosis Factor Alpha , Eur Urol doi:10.1016/j.eururo.2009.01.030]*. A possible explanation might be that tadalafil alone showed cross-reactivity with PDE 11 for which the exact role in the tissues still has to be explained.

Although MPO and the products of its activity are associated with general atherosclerosis, the study of their involvement in erectile dysfunction is only in its initial stages.

This data provides new possibilities for the understanding of the aetiology of erectile dysfunction and probably for the introduction of new treatments.