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TESI DI DOTTORATO DI RICERCA

OBESITY AND METABOLIC SYNDROME: PLASMA LIPOPROTEINS ALTERATIONS

BIO 10

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INTRODUCTION

1. THE METABOLIC SYNDROME

The metabolic syndrome has received increased attention in the past few years. It consists of multiple, interrelated risk factors of metabolic origin that appear to directly promote the development of atherosclerotic cardiovascular disease (ASCVD). This constellation of metabolic risk factors is strongly associated with type 2 diabetes mellitus or the risk for this condition. The metabolic risk factors consist of atherogenic dyslipidemia, elevated blood pressure, elevated plasma glucose and visceral obesity.

Several different sets of criteria have been proposed during the past decade for diagnosis of the metabolic syndrome. In 2001, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) proposed a simple set of diagnostic criteria based on common clinical measures including waist circumference, triglycerides, HDL-C, blood pressure, and fasting glucose level ((al.)1). The presence of defined abnormalities in any 3 of these 5 measures constitutes a diagnosis of the metabolic syndrome (Table 1).

The ATP III criteria for the metabolic syndrome have been widely used in both clinical practice and epidemiological studies. The criteria also have the advantage of avoiding emphasis on a single cause.

In 2005, the International Diabetes Federation (IDF) has proposed a new classification that includes central obesity as an essential element for the diagnosis, changing the parameters of waist circumference (Table 1).

Table 1. Diagnostic criteria for metabolic syndrome

NCEP-ATPIII	IDF

Waist circumference
• ≥ 102 cm in men
• ≥ 88 cm in women
Triglycerides ≥ 150 mg/dl or drug treatment
HDL cholesterol
• < 40 mg/dl in men
• < 50 mg/dl in women
Blood pression
• ≥ 130 mm Hg systolic
  Or
• ≥ 85 mm Hg diastolic
  Or drug treatment for hypertension
Fasting glucose ≥ 100 mg/dl or previous diagnosis of type 2 diabetes
	Waist circumference
	• ≥ 94 cm in men
	• ≥ 80 cm in women
	Triglycerides ≥ 150 mg/dl or drug treatment
	HDL cholesterol
	• < 40 mg/dl in men
	• < 50 mg/dl in women
	Blood pression
	• ≥ 130 mm Hg systolic
	  Or
	• ≥ 85 mm Hg diastolic
	  Or drug treatment for hypertension
	Fasting glucose ≥ 100 mg/dl or previous diagnosis of type 2 diabetes

Prospective population studies show that the metabolic syndrome confers an 2-fold increase in relative risk for ASCVD events, and in individuals without established type 2 diabetes mellitus, an 5-fold increase in risk for developing diabetes as compared with people without the syndrome. This finding implies that the metabolic syndrome imparts a relatively high long-term risk for both ASCVD and diabetes (1).

At present, it is not clear whether the metabolic syndrome has a single cause, and it appears that it can be precipitated by multiple underlying risk factors. The most important of these underlying risk factors are abdominal obesity and insulin resistance.

The abdominal visceral adipose tissue is a source of cytokines and adipokines that influence interactions between the immune system and the vascular wall, inducing a state of chronic inflammation and increased oxidative stress (2). When the picture is complicated with metabolic changes on carbohydrate and lipid metabolism, the risk factors for cardiovascular and cerebrovascular disease, already high in a state of uncomplicated obesity, increased. It is still not clear what is the alteration of departure, but it seems increasingly clear that the single alteration should be able to influence each other defining a complex metabolic condition as that of the metabolic syndrome.
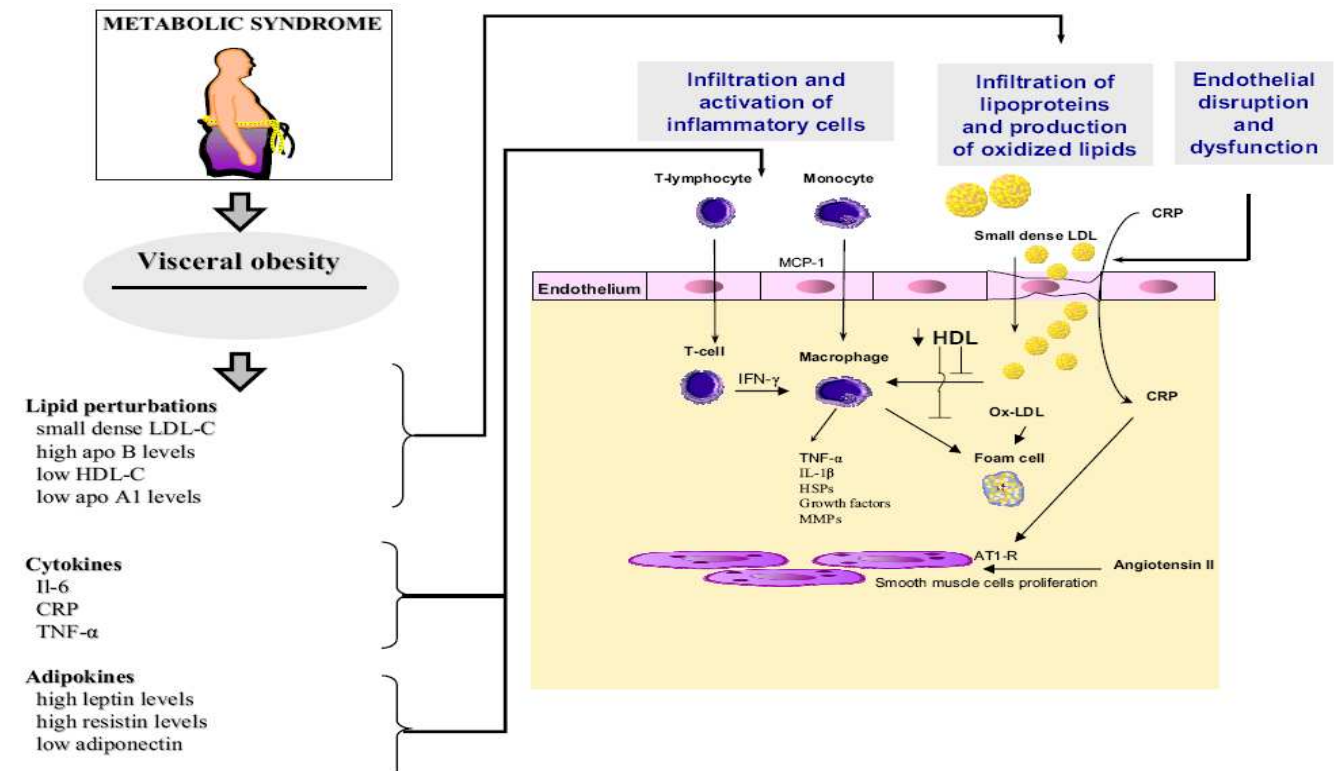
2. LIPOPROTEINS BIOCHEMISTRY

Cholesterol and triacylglycerol are transported in blood as lipoproteins. Lipoproteins are generally spherical particles, with a surface layer composed of phospholipid with the fatty acids oriented toward the core of the particle. Included in this phospholipid layer are specific proteins known as apolipoproteins and free cholesterol. The core of the lipoprotein particles is made up of cholesteryl ester and triacylglycerol molecules.

The classification of serum lipoproteins has evolved historically through several phases corresponding with the development of different laboratory methodologies.

With the arrival of the analytical ultracentrifugation in the 1940s, lipoproteins were classically separated into four major classes designated as chylomicrons (exogenous triacylglycerol-rich particles of d <0.94 g/ml), very low-density lipoproteins (VLDL, endogenous triacylglycerol- rich particles of d=0.94–1.006 g/ml), LDL (cholesteryl ester-rich particles of d=1.006– 1.063 g/ml), and HDL (particles containing approximately 50% protein of d=1.063– 1.21 g/ml). With subsequent improvements to the ultracentrifugation techniques, further heterogeneity was detected within each of those major lipoprotein classes; this resulted in the need for further subdivision into several

density subclasses such as HDL2a (d=1.10–1.125 gml_1), HDL2b (d=1.063– 1.10 gml_1), and HDL3 (d=1.125–1.21 gml_1). There is no doubt that the separation of lipoproteins by ultracentrifugation has been essential for the advances in this field.

2.1 SYNTHESIS AND CATABOLISM OF LIPOPROTEINS

2.1.1 Metabolism of Lipoproteins Carrying Exogenous Lipids

Dietary fats absorbed in the intestine are packaged into large, triacylglycerol-rich chylomicrons for delivery through the bloodstream to sites of lipid metabolism or storage. These lipoproteins interact with lipoprotein lipase (LPL) and undergo lipolysis, forming chylomicron remnants. The major sites of LPL activity are adipose tissue, skeletal muscle, the mammary gland, and the myocardium. In these sites, the fatty acids from the trcacylglycerols are used for storage, oxidation, or secretion back to the circulation. The triacylglycerol-depleted particles resulting from the lipolysis, known as chylomicron remnants, pick up apo E and cholesteryl ester from HDL and are rapidly taken up by the liver via a process mediated by the apo E receptor. This is a fast process and chylomicron particles are not usually present in the blood after a prolonged fasting period. The occurrence of chylomicronemia can be easily detected by the presence of a creamy supernatant floating on top of the plasma or serum kept several hours at 4 °C.

2.1.2 Transport of Endogenous Lipids

The liver cell secretes triacylglycerol-rich VLDL, which can be converted first to intermediate-density lipoprotein (IDL) and then to LDL through lipolysis by a mechanism similar to that described for chylomicrons. The excess surface components are usually transferred to HDL, and the triacylglycerol-depleted VLDL becomes an IDL. Some of these particles may be taken up by the liver via an apo E receptor, whereas others are further depleted of triacylglycerols, becoming cholesteryl ester-enriched particles known as LDL, which contain apo B as their only apolipoproteins. Consumption of fat-rich meals or glucose enhances VLDL production. Some primary causes of elevated VLDL or IDL levels are familial endogenous hypertriglyceridemia (type IV according to Fredrickson's classification) and familial dysbetalipoproteinemia (type III hyperlipidemia). Genetic mutations at the apo E gene locus are responsible for the type III phenotype. Some secondary causes for elevated VLDL levels are obesity, diabetes mellitus, alcohol

consumption, as well as the use of high doses of certain drugs (e.g., thiazide diuretics and estrogens). The presence of elevated levels of IDL has been associated with an increased atherosclerotic risk. LDL particles are major carriers of cholesteryl ester in the blood. An LDL receptor that recognizes apo B-100 and apo E, but not apo B-48, allows the liver and other tissues to catabolize LDL. High-fat and high-cholesterol diets can decrease the activity of the LDL receptor, leading to increased levels of circulating LDL. These particles supply cholesterol to cells in the periphery for synthesis of cell membranes and steroid hormones. Modified or oxidized LDL can also be taken up by the scavenger receptor on macrophages in various tissues, including the arterial wall. This process is a potential initiator of foam cell formation and atherosclerosis. Several LDL subclasses have been identified using gradient gel electrophoresis. Large, less dense LDL particles are commonly found in premenopausal women and men at low risk for CHD, whereas the small, more dense particles have been associated with a significant increased risk for myocardial infarction. The distribution of these particles appears to have a significant genetic component modulated by age and environmental factors.

2.1.3 Reverse Cholesterol Transport

HDL is synthesized by both the liver and the intestine. Its precursor form is discoidal in shape and matures in circulation as it picks up unesterified cholesterol from cell membranes and other lipids (phospholipid and triacylglycerol) and proteins (AI, E, and C apolipoproteins) from triacylglycerolrich lipoproteins (chylomicron and VLDL) as these particles undergo lipolysis. The cholesterol is esterified by the action of the lecithin–cholesterol acyltransferase (LCAT) and the small HDL3 particle becomes a larger HDL2 particle. The esterified cholesterol is either delivered to the liver or transferred by the action of cholesteryl ester transfer protein (CETP) to other lipoproteins (such as chylomicron, VLDL remnants, or LDL) in exchange for triacylglycerols. This cholesterol may then be taken up by the liver via receptors specific for these lipoproteins, or it can be delivered again to the peripheral tissues. The triacylglycerol received by HDL2 is hydrolyzed by hepatic lipase and the particle is converted back to HDL3, completing the HDL cycle in plasma. In the liver, cholesterol can be excreted directly into bile, converted to bile acids, or reutilized in lipoprotein production. Several genetic disorders have been identified associated with low levels or total deficiency of HDL(4).

3. THE ROLE OF OXIDIZED LIPOPROTEINS LDL IN ATHEROGENESIS

LDL lipoproteins are composed of a core enriched in hydrophobic triglycerides and cholesterol ester molecule, enclosed by an envelope of phospholipids and unesterified cholesterol and lipoprotein ApoB 100 (5).

The lipoproteins LDL are generally divided into three main subclasses based on density: LDL1 and LDL2 (density 1018-1030 g / mL), LDL3 (1030-1040 g / mL), LDL4 and LDL5 (1040-1065 g / mL). The small, dense LDL can penetrate more easily into the subendothelial space of the vascular wall and are more susceptible to oxidation (6). Several components of LDL, including ApoB, phospholipids, cholesterol and unsaturated fatty acids may be subject to oxidative stess (7). Vitamin E is the principal fat soluble antioxidant that protects lipoproteins, but when it is consumed, the chain reaction continues and develops degradation products as the malonilaldeide (MDA) (8).

Aldehydes can form Schiff bases with amino groups of lysine residues, creating cross-links between lipids and proteins or between lipid molecules. These changes alter the protein fraction and reduce the affinity of LDL for its receptor, but increases the affinity for the scavenger receptor of macrophages, through which oxidized LDL exert their atherogenic action. Macrophages incorporate oxidized LDL, contributing to foam cell formation and playing a key role in cellular events that lead to the development of atherosclerotic lesions. The oxidation of lipoproteins in the plasma seems to occur with difficulty due to the presence of high concentrations of antioxidants and proteins that chelate metal (9).

It has been suggested that oxidized LDL are generated in the vessel wall and spread into the bloodstream after plaque rupture, increasing the permeability of plaque and leading to the ischemic and inflammatory damage (10). Several studies have found wide variation in susceptibility of LDLs from different individuals to oxidation ex vivo, though it has yet to be demonstrated convincingly that such variations are associated with the risk of atherosclerosis (9). Vitamin E, the major antioxidant carried in LDL, may be one factor that influences the susceptibility of LDL to oxidation. Increasing the vitamin E content of lipoproteins in vitro or in vivo (by dietary supplementation) increases the lag phase for initiation of LDL oxidation under certain conditions.

Other lipoprotein characteristics such as size and density also influence the extent of oxidation. Smalldense LDL is more susceptible to oxidation than large buoyant LDL, and increased levels of small-dense LDLs are seen in various conditions that increase the risk of vascular disease, including diabetes, combined hyperlipidemia, and familial dyslipidemic hypertension. The increased

susceptibility to oxidation may relate to these particles' increased content of triglycerides, polyunsaturated fatty acids, and/or decreased vitamin E content. (9)

4. HDL LIPOPROTEIN

The high-density lipoprotein (HDL) have been described for the first time in the early 50's with the ultacentrifugation density gradient method to separate the main classes of lipoproteins. Important epidemiological studies, most notably the Framingham Heart Study, have demonstrated a powerful inverse relationship between cardiovascular disease and plasma concentrations of HDL cholesterol (10). Low levels of HDL cholesterol (<40 mg / dl in women and <50 mg / dl in males) were identified as independent risk factors for cardiovascular disease, both in diabetics, both in non-diabetic subjects (11).

4.1 Functional HDL: structure, composition, and heterogeneity

Functional plasma HDL are spherical or discoidal particles of high hydrated density (1.063-1.21 g/ml) due to elevated protein content (>30% by weight) compared with other lipoproteins (12;13). Discoidal HDL are small particles consisting primarily of apoA-I embedded in a lipid monolayer constituted of PL and free cholesterol (14;15). Spherical HDL are larger and additionally contain a hydrophobic core formed by cholesteryl esters (CE) and small amounts of TG. ApoA-I (molecular mass 28 kDa) is the major structural HDL apolipoprotein and accounts for ~70% of total HDL protein, whereas the second major HDL apolipoprotein, apoA-II, represents ~20%. Minor HDL protein components (typically <10% of the HDL protein moiety) include apoE, apoA-IV, apoA-V, apoJ, apoC-I, apoC-II, and apoC-III (13). In small discoidal HDL, two molecules of apoA-I adopt a "double belt" orientation with their helixes oriented parallel to the plane of the disc and perpendicular to the lipid acyl chains in such a way that they wrap around the lipid bilayer disc forming two stacked rings in an antiparallel orientation (17); furthermore, apoA-I molecules appear to slide in relation to each other between two stable conformations (18). Plasma HDL particles also carry enzymes involved in lipid metabolism, including lecithin/cholesterol acyltransferase (LCAT), enzymes with plausible antioxidative activities, such as platelet-activating factor-acetyl hydrolase (PAF-AH, also called lipoprotein-associated phospholipase A_2), paraoxonase 1 (PON1) and glutathione selenoperoxidase (GSPx), and other proteins and peptides, such as serum amyloid A (SAA), a major positive acute-phase reactant, α-1-antitrypsin, or amyloid-β, the principal constituent of senile plaques in Alzheimer's disease (17).

Heterogeneity in the physicochemical properties of normal functional HDL in healthy normolipidemic subjects (18).

Plasma HDL particles are highly heterogeneous in their physicochemical properties, metabolism, and biological activity (13). Such heterogeneity results from differences in relative contents of apolipoproteins and lipids in HDL and is intimately related to the amphipathic helical structure of human apoA-I (19); these helixes possess a hinge domain that allows apoA-I to switch between two conformations corresponding to HDL particles of different size. When fractionated by ultracentrifugation, human HDL is typically separated into two major subfractions, HDL2 (*d* 1.063-1.125 g/ml) and HDL3 (*d* 1.125-1.21 g/ml) (20). Given the complexity of HDL particle heterogeneity, small, dense HDL will be defined for present purposes as lipid-poor and protein-rich discoidal and spherical HDL particles of small size (≤9 nm), low molecular mass (≤200 kDa), and high density (1.125-1.24 g/ml). Depending on the fractionation method, small, dense HDL may include HDL3a, 3b, and 3c and very high-density lipoprotein separated by ultracentrifugation and pre-β-HDL separated by gradient gel electrophoresis.

The clinical relevance of circulating levels of individual HDL subfractions to atherosclerosis and CV disease is, however, unclear Furthermore, plasma levels of either large (21) or small (22) HDL were reported to be associated with the progression of coronary atherosclerosis.

4.2 Metabolism

Spherical plasma HDL are mature particles generated by intravascular processes from lipid-free apoA-I or lipid-poor pre-β-HDL (Fig. 1) (23). These small HDL precursors are produced as nascent HDL by the liver or intestine, are also released as surface fragments from lipolysed TG-rich lipoproteins (VLDL and chylomicrons), and finally may be generated during the interconversion of HDL3 and HDL2. Small nascent HDL are unstable and readily acquire lipids (24); their initial lipidation occurs at cellular membranes via the ATP-binding cassette transporter (ABC) A1-mediated efflux of cholesterol and PL from cells (25). ABCA1 is a major player in HDL metabolism; indeed, genetic defects in ABCA1 as occur in Tangier disease may result in low HDL-C levels, with cholesterol accumulation in peripheral tissues and premature atherosclerosis (25).
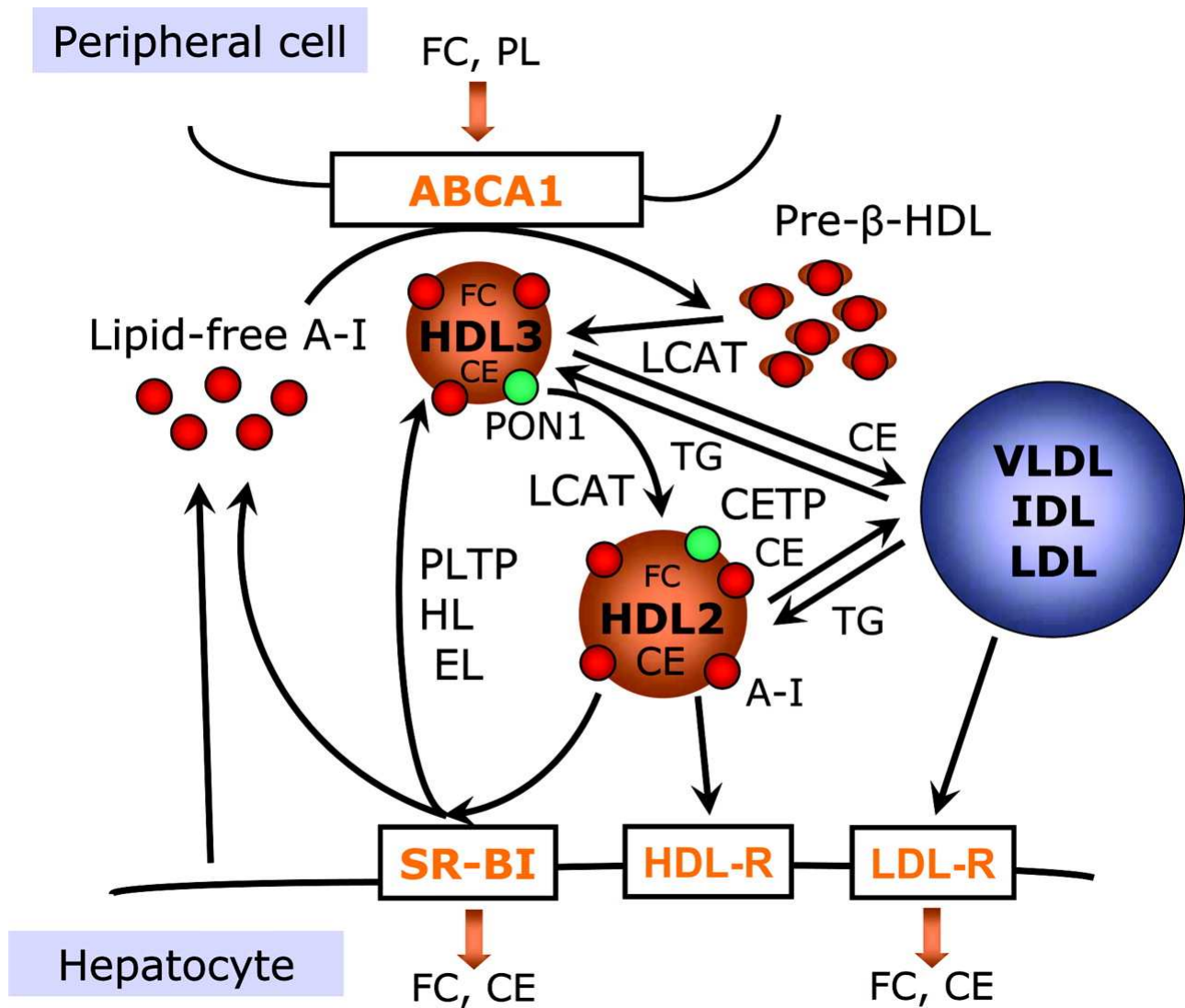
Fig. 1.

Intravascular HDL particle remodeling and metabolism in normolipidemia. Spherical plasma HDL are generated from lipid-free apoA-I or lipid-poor pre-β-HDL, which are produced as nascent HDL by the liver or intestine but can also be released as surface fragments from lipolysed TG-rich lipoproteins and/or during the interconversion of HDL3 and HDL2. Initial lipidation of small nascent HDL occurs at cellular membranes via the ABCA1-mediated efflux of cholesterol and PL from cells. Subsequent LCAT-mediated cholesterol esterification generates large spherical HDL2 particles, which undergo further remodeling via particle fusion and surface remnant transfer mediated by PLTP. Large HDL2 can be converted in turn to small HDL3 upon CETP-mediated transfer of CE from HDL to apoB-containing lipoproteins, upon SR-BI-mediated selective uptake of CE by the liver and steroidogenic organs, and HL- and endothelial lipase-mediated hydrolysis of TG. When CETP-mediated transfer of CE occurs between HDL and TG-rich lipoproteins, TG-rich HDL are generated, which can be further hydrolyzed by HL to small, TG-rich HDL particles. The concerted action of CETP and HL promotes reduction in HDL size, formation of lipid-poor HDL particles, and shedding from HDL of lipid-free apoA-I, which can interact with ABCA1 in the next lipidation cycle. HDL lipids are catabolized either separately from HDL proteins by selective uptake or via CETP transfer or as holoparticles together with HDL proteins primarily in the liver via uptake through LDL receptors for apoE-containing HDL and through hitherto unidentified receptors for HDL holoparticles. EL, endothelial lipase; FC, free cholesterol; HDL-R, HDL holoparticle receptor; LDL-R, LDL receptor.

Subsequent LCAT-mediated esterification of cell-derived cholesterol generates large spherical HDL particles with a neutral lipid core of CE and TG ; such particles undergo further remodeling via

particle fusion and surface remnant transfer mediated by phospholipid transfer protein (PLTP). Large HDL2 can be converted in turn to small HDL3 upon cholesteryl ester transfer protein (CETP)-mediated transfer of CE from HDL to apoB-containing lipoproteins, upon scavenger receptor type BI (SR-BI)-mediated selective uptake of CE by the liver and steroidogenic organs and hepatic lipase (HL) and upon endothelial lipase-mediated hydrolysis of core TG (26). When CETP-mediated transfer of CE occurs between HDL and TG-rich lipoproteins, TG-rich HDL are generated, which can be further hydrolyzed by HL to small, TG-rich HDL particles (27). The concerted action of CETP and HL promotes reduction in HDL size, formation of lipid-poor HDL particles and shedding from HDL of lipid-free apoA-I, which can interact with ABCA1 in the next lipidation cycle (28). HDL lipids are catabolized either separately from HDL proteins by selective uptake or via CETP transfer or as holoparticles primarily in the liver, via uptake through LDL receptors for apoE-containing HDL and through hitherto unidentified receptors for HDL holoparticles.

4.3 Biological Activities

HDL particles possess multiple antiatherogenic. The central role of HDL in cellular cholesterol efflux and RCT is considered to form a basis for the capacity of HDL to attenuate atherogenesis. However, compelling evidence has emerged that additional dimensions of the antiatherogenic action of HDL may be of major physiological and pathological relevance (29).

4.3.1 Cholesterol Efflux Capacity

The cholesterol efflux capacity of HDL particles is related to their ability to remove cholesterol from membranes of peripheral cells and particularly macrophages and foam cells via interaction with the ABCA1 and ABCG1 transporters and/or SR-BI receptor. Apolipoprotein-mediated lipid efflux involves specific interactions with membrane proteins, desorption of membrane lipids from caveolae, lipidation of lipid-free apolipoproteins and production of small, lipid-poor HDL (26). Lipid-free apolipoproteins remove cholesterol and PL from macrophages, aortic smooth muscle cells, and normal human skin fibroblasts but not from fibroblasts of patients with Tangier disease (25). Defective ABCA1 transporter function in Tangier disease has provided clear evidence that ABCA1 has a central role in lipid efflux mediated by lipid-poor apolipoproteins. In support of this mechanism, apoA-I-mediated cholesterol efflux is severely decreased by inhibition of ABCA1 with either antisense oligonucleotides or pharmacological compounds but is increased by the

overexpression of ABCA1 (25). Thus, ABCA1 is a pivotal regulator of cellular cholesterol efflux and of the lipidation of apoA-I, a key step in formation of mature, spherical HDL particles.

ABCA1 has two highly conserved cytoplasmic ATP binding cassettes and two transmembrane domains, each of which consists of six membrane-spanning segments (30). It has been suggested that ABCA1 forms a channel within the plasma membrane through which cholesterol and PL are transferred ("flopped") from the inner to the outer leaflet of the plasma bilayer membrane et al. There the lipids may be picked up by lipid-free apolipoproteins or lipid-poor particles, which bind to ABCA1 (31).

In addition to ABCA1, there are several other sterol-regulated ABC transporters, including ABCG1 and ABCG4, which are involved in cholesterol efflux from macrophages to mature HDL2 and HDL3 particles (32). The relative quantitative importance of cholesterol efflux mediated by ABCA1 compared with ABCG1 in macrophages remains unclear.

In contrast to lipid-free apolipoproteins, lipid-containing HDL particles induce both specific and nonspecific forms of cholesterol efflux (26). Nonspecific cholesterol efflux can be also mediated by PL vesicles, synthetic cyclodextrins, albumin or partially proteolysed HDL; it is slow, unsaturable, and bidirectional and thus appears to occur by aqueous diffusion . It has been suggested that SR-BI mediates the bidirectional flux between mature HDL and plasma membranes through the binding of HDL particles and subsequent reorganization of lipids within cholesterol- and caveolae-rich domains in the plasma membrane . The PL content of HDL is an important determinant of such SR-BI-mediated cholesterol efflux (33). Finally, HDL-mediated cholesterol efflux from macrophages may be facilitated by apoE secretion (34). Indeed, macrophage-derived apoE can associate with HDL and improve its cholesterol acceptor properties.

Distinct cholesterol efflux properties of lipid-free and lipid-containing HDL are indicative of functional heterogeneity of HDL particles. Indeed, a decrease in the lipid content of HDL is generally thought to increase its capacity to remove cellular cholesterol; small, dense, lipid-poor, protein-rich HDL particles are therefore considered to represent more efficient cholesterol acceptors compared with their large, light, lipid-rich, protein-poor counterparts (26), thereby same authors suggest that lipid-free, rather than lipid-poor, apolipoproteins function as primary cholesterol acceptors (37). Lipid-free and/or lipid-poor HDL apolipoproteins induce cholesterol uptake via interaction with ABCA1; conversely, large, lipid-rich HDL particles appear to represent a better ligand for cellular uptake of CE mediated by SR-BI compared with small, lipid-poor, consistent with the role of these particles in RCT from peripheral cells to the liver (26,37).

4.3.2 Antioxidative Activity

HDL antioxidative activity is typically observed as inhibition of LDL oxidation by HDL; LDL is thought to represent the major physiological target of HDL antioxidative action in vivo (29). HDL is also able to inhibit generation of reactive oxygen species (ROS) in vitro under conditions of cell culture and in vivo in a rabbit model of acute arterial inflammation . In addition, inhibitory actions of HDL on LDL oxidation have been reported in vitro upon their coincubation and in vivo upon HDL injection. HDL potently protects both lipid and protein moieties of LDL and inhibits accumulation of various oxidation products in LDL, including oxidized PL and short-chain aldehydes (36,29).

The antioxidative activity of HDL is related to the presence of several apolipoproteins and enzymes with antioxidative properties in HDL particles. Apolipoproteins that possess antioxidative activity include apoA-I, apoE, apoJ, apoA-II, and apoA-IV. It appears that a major component of the antioxidative activity of HDL can be ascribed to apoA-I which can prevent and/or delay LDL oxidation by removing oxidized PL, including 1-palmitoyl-2(5-oxovaleroyl)-*sn*-glycero-3-phosphorylcholine and 1-palmitoyl-2-glutaroyl-*sn*-glycero-3-phosphorylcholine, from LDL and from arterial wall cells (37). The capacity of apoA-I to remove oxidized lipids is not specific for arterial wall cells, because similar effects have been reported for erythrocytes (36). Circulating HDL accumulate LOOH and have been proposed to function as a "sink" for oxidized lipids (39), ensuring their efficient elimination from the circulation through the liver.

ApoE possesses established antiatherosclerotic activity, which is normally ascribed to its lipid transport properties . However, the action of apoE goes beyond such activity. Indeed, apoE possesses distinct antioxidative properties and can promote regression of atherosclerosis independently of lowering plasma cholesterol levels (40). HDL-associated apoJ can inhibit oxidation of LDL by artery wall cells ; in addition, apoJ is cytoprotective at low physiological levels. The beneficial actions of apoJ may be related to its ability to maintain integrity of membrane and lipoprotein lipids via its hydrophobic-binding domains (29). Antioxidative properties have also been reported for apoA-II and apoA-IV. The capacity of apoA-II to protect LDL from oxidation is, however, questionable, given the fact that overexpression of human apoA-II in dyslipidemic mice accelerates atherosclerosis, increases aortic accumulation of oxLDL, and reduces antioxidative activity of HDL. Such proatherogenic actions of apoA-II may be related to the displacement of antiatherogenic apoA-I and PON1 by apoA-II from HDL particles (41). Finally, HDL is able to function as a preventive antioxidant through its capacity to bind transition metal ions, which in free

form are potent catalyzers of LDL oxidation. Intriguingly, plasma HDL carry amyloid-β peptide, a major component of senile neuritic plaques and a strong chelator of transition metals (29).

Major HDL enzymes possessing antioxidative activity are PON1, PAF-AH, LCAT, and GSPx (29). PON1 is a component of HDL that is thought to hydrolyze LDL-derived short-chain oxidized PL once they are formed (42). PON1 is anchored to lipids via its hydrophobic N terminus; the association of PON1 with HDL is a prerequisite for maintaining normal serum activity of the enzyme. HDL provides the optimal physiological acceptor complex for PON1, in terms of both stimulating enzyme secretion and stabilizing the secreted peptide ; PON1 interaction with apoA-I is critical for enzyme stability . HDL and, less efficiently, VLDL but not LDL promote PON1 secretion from cells; the differences between these lipoproteins are related to differences in their lipid composition (43).

PAF-AH and LCAT can also hydrolyze LDL-derived short-chain oxidized PL; the relationship between the hydrolyzing activities of PON1, PAF-AH, and LCAT toward oxidized PL remains unclear. Recent data question the ability of PON1 to hydrolyze oxidized PL and suggest that PAF-AH, rather than PON-1, is the oxidized PL hydrolase in HDL. Consistent with this conclusion, HDL-associated PAF-AH is thought to play an antiatherogenic role, in contrast to the LDL-associated. Indeed, local arterial expression of PAF-AH reduces accumulation of oxLDL and inhibits inflammation, shear stress-induced thrombosis, and neointima formation in balloon-injured carotid arteries of nonhyperlipidemic rabbits (44).

The antioxidative activity of PON1 purified from human serum has recently been ascribed to the presence of detergents or some other unidentified proteins. Interestingly, PON1 has been reported to catalyze the hydrolysis of a variety of lactones, including homocysteine thiolactone, suggesting that its native activity is as a lactonase. Plasma levels of homocysteine are a strong CV risk factor; by detoxifying homocysteine thiolactone, PON1 could protect against homocysteinylation, a post-translational modification of proteins associated with attenuated biological activity and a potential contributing factor to atherosclerosis (38).

In addition, HDL-associated PON1 enhances cholesterol efflux from macrophages via increased HDL binding mediated by ABCA1 PON1-induced cellular accumulation of lysophosphatidylcholine, which stimulates cholesterol efflux via the ABCA1 pathway, may account for this effect. One can hypothesize that both lactonase activity and an RCT-related mechanism may contribute to the antiatherosclerotic effects of PON1 observed in vivo (45).

Another HDL enzyme, GSPx, can reduce LOOH to corresponding hydroxides and thereby detoxify them. LOOH-reducing activity mediated by Met residues of apoA-I and apoA-II has also been reported. Finally, upon HDL oxidation with peroxynitrite, apoA-I increases generation of PL core

aldehydes that are subsequently hydrolyzed by HDL-associated enzymes, such as PAF-AH and/or PON1, with formation of lysophospholipids. Such a PAF-AH/PON1-coupled protective function of apoA-I can effectively divert proatherogenic LOOH to less harmful products (36; 29).

Apolipoproteins and enzymes with antioxidative activities are nonuniformly distributed across HDL subfractions. In vivo PON1 is preferentially associated with large HDL but can be displaced to small, dense particles upon ultracentrifugation (15,16). The size and shape of HDL seem to be critical for PON1 binding (38). By contrast, apoJ is associated with a subset of small HDL, which also contains PON1 (41). Similarly, LCAT activity (18), PAF-AH activity (18), and apoA-IV (41) are enriched in small, dense HDL isolated by ultracentrifugation. As a consequence, HDL particles are heterogeneous in their antioxidative activity. Under mild oxidative stress induced by an azo initiator 2,2′-azobis-(2-amidinopropane) hydrochloride or Cu^{2+}, the antioxidative activity of HDL subfractions isolated by density gradient ultracentrifugation against LDL oxidation increases with increment in density in the order: HDL2b < HDL2a < HDL3a < HDL3b < HDL3c, thereby establishing that small, dense HDL act as potent protectors of LDL from oxidative stress. Similarly, HDL3 is a more potent protector of LDL from in vitro oxidation compared with HDL2. The antioxidative activity of small, dense HDL is related to the inactivation of proatherogenic products of LDL lipid peroxidation, primarily LOOH. Mechanistically, this activity may arise from synergy in inactivation of oxidized lipids by enzymatic (hydrolysis) and nonenzymatic (physical removal) mechanisms, in part reflecting distinct intrinsic physicochemical properties of the small, dense HDL3c subfraction (15).

The relative importance of HDL antioxidative activity in the overall cardioprotective effect of HDL compared with other biological actions remains indeterminate.

4.3.3 Anti-Inflammatory Activity

The anti-inflammatory activity of HDL is illustrated by the ability of HDL to decrease cytokine-induced expression of adhesion molecules on endothelial cells and to inhibit monocyte adhesion to these cells. HDL efficiently inhibit expression of the vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin in vitro induced by tumor necrosis factor-α (TNF-α), interleukin (IL)-1, or endotoxin. Moreover, this potent anti-inflammatory activity observed in vitro can be translated into inhibition of adhesion molecule expression and a decrease in neutrophil infiltration in the arterial wall by reconstituted HDL (rHDL) in a rabbit model of acute arterial inflammation (46). The ability of HDL to inhibit adhesion molecule expression may be related to the presence of apoA-I, apoA-II, apoA-IV, and/or distinct molecular species of PL, including

sphingosine-1-phosphate (S1P) and sphingosylphosphorylcholine . The anti-inflammatory action of HDL involves inhibition of TNF-α-stimulated activation of sphingosine kinase (47). In addition, HDL attenuate IL-6 production in endothelial cells exposed to proinflammatory stimuli, such as TNF-α or endotoxin (48).

The anti-inflammatory action of HDL also involves hydrolysis of oxidized lipids by HDL-associated enzymes (PAF-AH and PON1) and is mechanistically similar to the antioxidative activity of HDL (36,49). Oxidized PL possess potent proinflammatory activities and can trigger arterial inflammation. Inactivation of oxidized lipids by HDL may be associated with decreased expression of adhesion molecules and decreased macrophage adhesion to endothelial cells (29).

Direct interaction of apoA-I with T lymphocytes, which can block subsequent activation of monocytes by lymphocytes, represents another plausible mechanism of HDL anti-inflammatory action . In addition, apoA-I has been reported to diminish neutrophil activation in vitro. The potential heterogeneity of HDL anti-inflammatory activity remains poorly characterized. HDL3 has been reported to be superior to HDL2 in terms of its capacity to inhibit vascular cell adhesion molecule-1 expression in endothelial cells (17).

4.3.4 Antiapoptotic, Vasodilatory, Antithrombotic, and Anti-Infectious Activities

Other antiatherogenic activities of HDL include antiapoptotic and vasodilatory actions, mitogenic activity in endothelial cells, attenuated platelet activation, and anticoagulant and anti-infectious activitie.

HDL potently inhibit apoptosis in endothelial cells induced by oxLDL or TNF-α ; this effect is paralleled by decreased intracellular generation of ROS and diminished levels of apoptotic markers, suggesting that it can be related to the intracellular antioxidative actions of HDL or HDL components. Indeed, HDL contain bioactive lysophospholipids, including S1P, a potent antiapoptotic agent, which may mediate the antiapoptotic effect of HDL via increased NO production.   Similarly, HDL vasodilatory activity may be related to the stimulation of NO release by endothelial cells mediated by intracellular Ca^{2+} mobilization and phosphorylation of NOS upon association with apoA-I (47). Such activation of NO production involves HDL binding to SR-BI with a subsequent increase in intracellular ceramide levels (8). Furthermore, HDL can stimulate production of prostacyclin, which possesses potent vasorelaxing activity. S1P may be equally important for mitogenic effects of HDL in endothelial cells and for the inhibitory action of HDL on the migration of vascular smooth muscle cells (49).

Similarly, increased production of NO may form a basis for the inhibitory action of HDL on platelet aggregation . The antithrombotic activity of HDL is observed as inhibitory actions on factors that promote blood coagulation (47). Mechanistically, this effect may be related to the presence of cardiolipin and phosphatidylethanolamine, two minor anionic PL with potent anticoagulant properties that are enriched in the HDL fraction (50). In addition, HDL acts via its protein moiety to enhance the anticoagulant activity of protein S and activated protein C (47).

Finally, HDL play a major role in the binding and clearance of circulating endotoxin to the bile and thereby inhibit endotoxin-induced cellular activation, resulting in potent anti-infectious activity (51).

The potential heterogeneity of these antiatherogenic activities among HDL particles is indeterminate.

4.4 Functionally Defective High-Density Lipoprotein in Dyslipidemic and Inflammatory States

HDL is known to undergo dramatic modification in structure and composition as a result of the concerted actions of the acute-phase response and inflammation. The close association between inflammation, oxidative stress, dyslipidemia, and atherosclerosis suggests that such HDL alterations play a significant role in disease progression. As a result, HDL particles progressively lose normal biological activities and acquire altered properties. Such altered HDL particles have been termed "dysfunctional HDL", and HDL has been proposed to possess "chameleon-like properties. It is essential to emphasize that the degree of loss of normal HDL function compared with the absence of this function depends on the assay used to characterize HDL functionality (38).

4.4.1Altered High-Density Lipoprotein Composition and Enzymatic Activities in Dyslipidemic and Inflammatory States

4.4.1.1 Apolipoproteins

Both the plasma levels and apolipoprotein content of HDL can be significantly altered during the acute phase as well as during acute and chronic inflammation. Levels of apoA-I and apoA-II decrease, whereas those of apoA-IV, apoA-V, apoJ, and apoE increase. The decrease in HDL apoA-

I levels in inflammatory states is related to both decreased apoA-I synthesis in the liver and apoA-I replacement in HDL particles by SAA (Fig.2). In the circulation, SAA does not exist in a free form, it is usually associated with HDL. Elevated plasma levels of SAA are accompanied by elevated levels of lipid-free apoA-I, probably due to the dissociation of apoA-I from HDL (52,53).
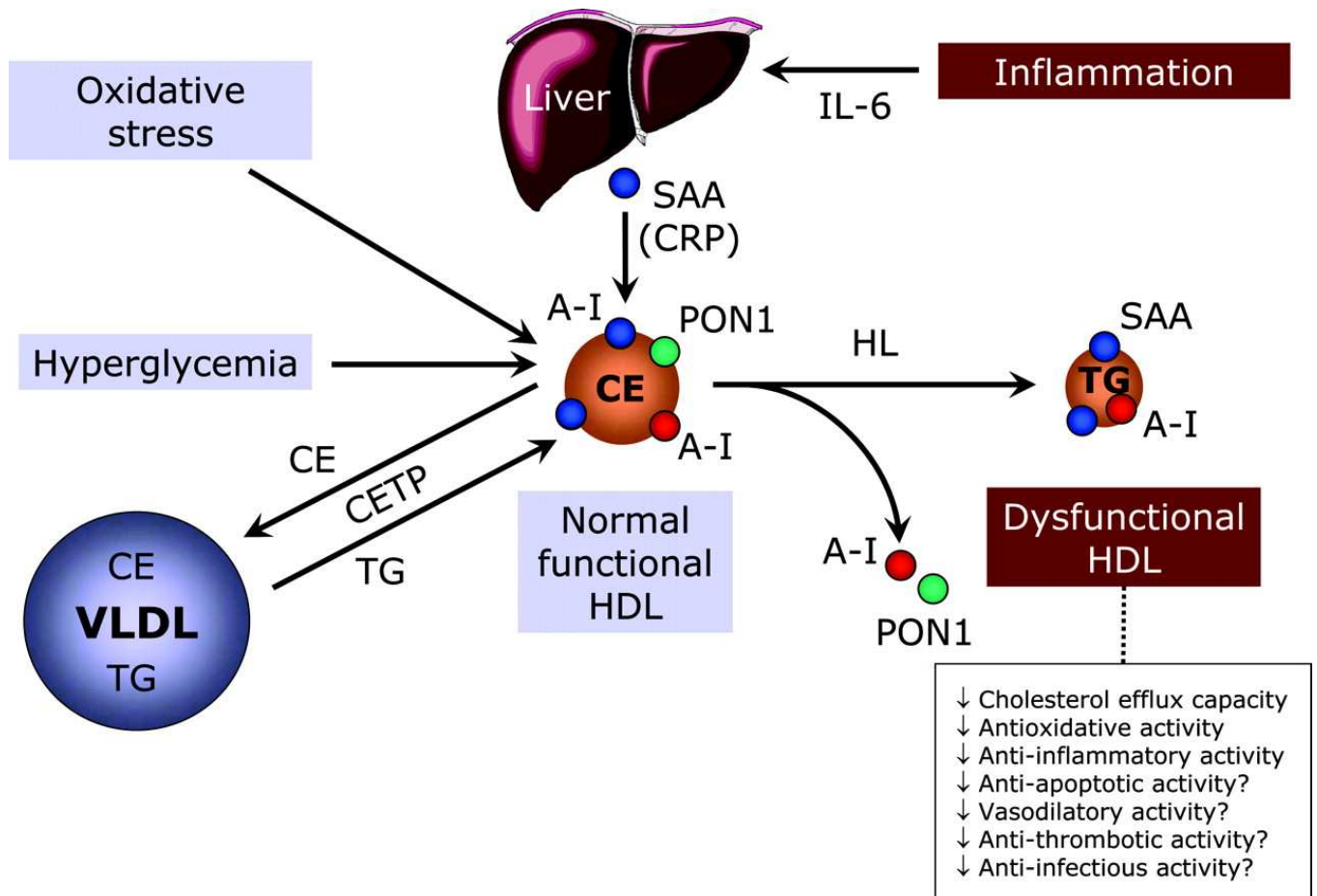
Fig. 2.

Abnormal metabolism and deficient biological activities of HDL in atherogenic dyslipidemias of metabolic disease. Chronic inflammation characteristic of metabolic disease, such as MetS and type 2 diabetes, is associated with elevated plasma levels of IL-6. As a result, the liver produces SAA, which replaces apoA-I and PON1 in HDL. Oxidative stress, hyperglycemia, and elevated activity of CETP are other important modulators of HDL function. Oxidative stress modifies specific amino acids in apoA-I, whereas hyperglycemia results in apoA-I glycation. CETP exchanges CE and TG between HDL and TG-rich lipoproteins, such as VLDL; as a result, HDL become enriched in TG. Such enrichment in TG induces conformational changes in apoA-I, which becomes less accessible for the interaction with other lipoproteins, including LDL, and cannot eliminate oxidized lipids from LDL. Subsequent HDL hydrolysis by HL produces small, dense HDL that are enriched in TG and in SAA and contain apoA-I in an incorrect conformation; such HDL possess deficient functionality compared with normal HDL particles.

Apart from its replacement by SAA, apoA-I can undergo other modifications in the circulation. Amino acid residues in apoA-I, such as methionine, cysteine, tyrosine, and lysine residues, can be selectively modified under the action of prooxidants secreted by arterial wall cells and nonenzymatically glycosylated in the presence of high levels of glucose (34). Oxidized amino acid residues, including chlorotyrosines, nitrotyrosines and oxidized lysine and methionine residues, are

present in apoA-I isolated from plasma and from human atherosclerotic lesions ; furthermore, the apoA-I content of chloro- and nitrotyrosines is increased in plasma of patients with CV disease. Myeloperoxidase was recently shown to bind to HDL within human atherosclerotic lesions, and biophysical studies reveal MPO binding occurs via specific interactions with apolipoprotein (apo) A-I. This likely facilitates the observed selective targeting of apoA-I for site-specific chlorination and nitration by MPO-generated reactive oxidants in vivo. One apparent consequence of MPO-catalyzed apoA-I oxidation includes the functional impairment of the ability of HDL to promote cellular cholesterol efflux via the ABCA1 system. Myeloperoxidase-mediated loss of the atheroprotective functional properties of HDL may thus provide a novel mechanism linking inflammation andoxidative stress to the pathogenesis of atherosclerosis (54).

4.4.1.2 Enzymes with Antioxidative and Anti-Inflammatory Properties

HDL-associated enzymes, including PAF-AH, PON1, and LCAT, can become dysfunctional and/or depleted under inflammatory conditions, in metabolic diseases involving low HDL levels (type 2 diabetes, MetS), and in premature CHD. HDL provides an amphipathic environment, where PON1 finds an optimal location to exert its activity. The complex HDL-PON1 is a respository for potentially toxic, hydrophobic components of plasma, notably oxidized lipids. Induction of the acute-phase response is associated with decreased PON1 activity, probably due to the replacement of PON1 by SAA (Fig.2) (29;36) . Furthermore, decreased PON1 activity may be caused by enzyme inactivation as a result of oxidation (55) and/or homocysteinylation and/or   glycation (46,64). Consistent with these observations, serum concentrations of PON1 are decreased in subjects with MetS (57) and in patients with type 1 and type 2 diabetes, who feature elevated levels of inflammation and oxidative stress. Serum PON1 activity decreases with age and is lower in subjects with MetS and low HDL-C and patients with type 2 diabetes and familial hypercholesterolemia (FH) compared with age-matched healthy control subjects. Moreover, low PON1 activity toward paraoxon has been reported to represent an independent risk factor for coronary events in men at high CV risk (57,58,59,60).

HDL-associated PAF-AH activity, expressed as a percentage of total serum PAF-AH activity, is lower in hypercholesterolemic patients than in control subjects. By contrast, LDL-associated PAF-AH activity is elevated, indicating a major redistribution of PAF-AH activity in plasma of dislipidemic individuals from apoA-I- to apoB-containing lipoproteins (61). Finally, LCAT activity is diminished under inflammatory conditions (38).

4.4.1.3 Lipid Components

Although apolipoproteins and enzymes are major determinants of altered HDL function, it is considerably influenced by changes in lipid content. HDL core enrichment in TG with CE depletion is the most frequent abnormality of HDL lipid composition (fig.2) and occurs in hypertriglyceridemic states, associated with decreased activity of LPL, decreased activity of HL, and/or decreased activity of LCAT. All these metabolic alterations are frequently observed in the acute phase and during inflammation (obesity is characterized by a low grade inflammation). In addition, HDL-TG content can be raised as a consequence of elevated CETP-mediated TG transfer from VLDL to HDL. Under such conditions, TG typically replace CE in the HDL core, resulting in a low CE/TG ratio and in a decrease in plasma HDL-C levels; another feature of the acute phase response (62). Interestingly, a similar elevation in HDL-TG, decrease in HDL-C and increase in inflammatory markers are observed in the postprandial phase. Human acute-phase HDL obtained from patients undergoing bypass surgery are enriched in TG and depleted of CE. Acute-phase HDL also contain elevated levels of nonesterified fatty acids (NEFA), lysophosphatidylcholines and isoprostanes compared with normal HDL; in addition, CE levels are decreased. Similarly, HDL3 from subjects with myocardial infarction are enriched in TG and depleted of PL. As a consequence of decreased LCAT activity, increased HDL concentrations of free cholesterol are frequently observed in inflammatory states; in addition, HDL free cholesterol is elevated in genetic LCAT deficiency (62).

4.5 Abnormal High-Density Lipoprotein Metabolism in Dyslipidemic and Inflammatory States

HDL metabolism is substantially altered in dyslipidemic states, including hypertriglyceridemia, hypercholesterolemia, mixed dyslipidemia and hypo- and hyperalphalipoproteinemia and also during infection and inflammation. As discussed above, hypertriglyceridemia is characterized by decreased levels of HDL-C and increased HDL-TG content due to the action of CETP. Such low HDL-C dyslipidemias associated with hypertriglyceridemia are characteristic of metabolic diseases associated with elevated CV risk, such as type 2 diabetes and MetS. Mechanisms leading to reduced plasma HDL-C levels and HDL particle numbers in hypertriglyceridemic states are as follows:

1) small HDL particles, which result from the intravascular lipolysis of TG-enriched HDL, are cleared more rapidly from the circulation;

2) TG-enriched HDL are intrinsically more unstable in the circulation, with apoA-I loosely bound;

3) lipolysis of TG-enriched HDL lower HDL particle numbers by causing apoA-I to be shed from HDL particles and cleared from the circulation;

4) dysfunctional LPL or reduced LPL activity contributes to the lowering of HDL levels by reducing the availability of surface constituents of TG-rich lipoproteins that sequester to the plasma pool of nascent HDL particles (63).

The CE/TG ratio therefore represents a critical factor in determining HDL particle stability and plasma residence time; HDL possessing decreased CE/TG ratios are less stable than normal particles (64). Importantly, a decrease in circulating HDL-C levels and an increase in TG levels are typical components of the acute-phase reaction (52). HDL metabolism critically depends on the activity of CETP. In metabolic diseases such as type 2 diabetes and MetS, elevated CETP activity results in increased CE transfer from HDL to TG-rich lipoproteins and in reciprocal TG transfer, producing TG-enriched HDL and decreasing HDL-C levels (fig.2). Conversely, CETP deficiency reduces the exchange of TG and CE between HDL and TG-rich lipoproteins and elevates HDL-C due to CE retention. As a consequence, increased CETP activity is thought to be proatherogenic in humans (65).

In hypercholesterolemia, abnormalities of HDL metabolism include moderate decreases in plasma apoA-I and HDL-C levels. HDL heterogeneity and particle profile largely reflect abnormalities in HDL metabolism. In the atherogenic dyslipidemias of MetS and type 2 diabetes, circulating levels of large, cholesterol-rich HDL decrease in parallel with decrease in HDL-C. By contrast, levels of small, dense, cholesterol-poor HDL particles and their content of apoA-I are rarely reduced in low HDL-C dyslipidemia (18). In obesity and insulin resistance, frequent features of both MetS and type 2 diabetes, plasma levels of large HDL decrease in parallel with those of HDL-C, whereas levels of small HDL do not. As a result, MetS, type 2 diabetes, obesity, and insulin resistance are all characterized by the prevalence of small, dense HDL (66).

Small, dense HDL also prevail in CHD patients. In male participants in the Framingham Offspring Study, subjects with CHD displayed higher levels of small particles. Similarly, subjects with new CV events possessed higher levels of small -HDL and lower levels of large HDL than subjects without such events in the Veterans Affairs HDL Intervention Trial (VA-HIT) study (35). CAD patients also display elevated levels of lipid-poor apoA-I . The increase in small HDL and decrease in HDL of intermediate size as measured by nuclear magnetic resonance are associated with CAD

severity in men admitted for diagnostic coronary arteriography. Small HDL also prevail in peripheral arterial disease (67).

4.6 Impaired High-Density Lipoprotein Biological Activities in Dyslipidemic and Inflammatory States

4.6.1 Cholesterol Efflux Capacity

Alterations in HDL composition and metabolism as occur in dyslipidemia and inflammation are intimately associated with impaired biological activities (Fig.2). However, data on HDL cholesterol efflux capacity in atherogenic dyslipidemia are conflicting. Some authors reported a diminished HDL capacity to deliver CE to hepatic cells through interaction with SR-BI as a result of HDL enrichment in TG (68).

By contrast, others have reported normal cholesterol efflux capacity of serum from hypertriglyceridemic subjects in Fu5AH cells, an observation that can be related to normal contents of HDL-PL, a key determinant of HDL-mediated efflux. Furthermore, HDL from hypertriglyceridemic CAD patients with low HDL-C levels possess a normal capacity to extract cholesterol from smooth muscle cells . Consistent with these results, TG-enriched HDL are not deficient in cholesterol efflux properties from cholesterol-loaded J774 macrophages (69).

The intrinsic cholesterol efflux capacity of HDL is considerably impaired during inflammation. Cellular cholesterol efflux is largely mediated by apoA-I-containing HDL particles; apoA-I replacement by SAA can therefore have a significant impact on efflux. Enrichment of HDL with SAA (up to high SAA contents of 86% of total HDL protein) results in increased HDL binding to, decreased cholesterol efflux capacity from, and increased selective CE uptake by macrophages Importantly, SAA selectively impairs cholesterol efflux properties of small, dense HDL3 particles. Less pronounced enrichment of HDL with SAA in vivo (up to 27% of total HDL protein) does not influence cholesterol efflux but enhances HDL binding to macrophages (70).

The presence of SAA increases both HDL affinity to and selective CE uptake by macrophages but reduces affinity to and CE uptake by hepatocytes. Decreased PL contents in inflammatory HDL constitute another factor that contributes to deficient HDL cholesterol efflux properties.Together, these changes lead to a significant shift in the HDL-mediated cholesterol transport from hepatocytes toward macrophages under acute-phase conditions. Biologically, such alterations may serve to redirect cholesterol to immune cells and to sites of injury and inflammation (71).

Abnormal lipid composition may also impair cholesterol efflux properties of HDL particles, as demonstrated by the diminished capacity of large, CE-enriched HDL2 isolated from subjects with homozygous CETP deficiency to accept cholesterol from lipid-loaded mouse peritoneal macrophages. Normalization of the lipid composition of such HDL, as a result of the transfer of excess CE to SR-BI-overexpressing cells, improves HDL cholesterol efflux capacity (71).

Oxidative modification represents another factor involved in the impairment of HDL cholesterol efflux capacity. In vitro oxidation of apoA-I by myeloperoxidase results in selective inhibition of ABCA1-dependent cholesterol efflux from macrophages (72). The cholesterol efflux capacity of apoA-I may be also impaired as a consequence of nonenzymatic glycosylation (56).

The central role of apoA-I in HDL-mediated cholesterol efflux is consistent with the deleterious role of apoA-I mutations. However, not all mutations in apoA-I lead to decreased cholesterol efflux capacity. ApoA-I Milano, a molecular variant of apoA-I, displays potent capacity for cholesterol efflux. Carriers of apoA-I Milano exhibit severe hypoalphalipoproteinemia but are not at increased risk for premature CHD (73).

Finally, the capacity of HDL particles to extract cholesterol from peripheral cells may be impaired as a result of alterations in cellular HDL receptors, primarily ABCA1 (74).

4.6.2 Antioxidative Activity

Recent evidence indicates that HDL particles are deficient in antioxidative activity in atherogenic dyslipidemias involving low HDL-C levels (25). Thus, the antioxidative activity of small, dense HDL subfractions against LDL oxidation induced by 2,2′-azobis-(2-amidinopropane) hydrochloride is significantly impaired in patients with MetS (up to -23%) and type 2 diabetes (up to -47%) . The impaired antioxidative activity of small, dense HDL in MetS and type 2 diabetes is intimately related to the concomitant presentation of hypertriglyceridemia, hyperinsulinemia, and insulin resistance, thereby suggesting that abnormalities in both lipid and glucose metabolism underlie the antioxidative deficiency of HDL particles (75). Furthermore, all HDL subfractions from subjects with a normotriglyceridemic, normocholesterolemic, normoglycemic low HDL-C phenotype display lower antioxidative activity (up to -43%) than their counterparts from normolipidemic control subjects. Interestingly, the intrinsic antioxidative activity of HDL particles is equally reduced in subjects with hyperalphalipoproteinemia associated with low HL activity and high HDL-TG content (76).

The antioxidative HDL deficiency in low HDL-C dyslipidemias of MetS and type 2 diabetes and in a normotriglyceridemic low HDL-C phenotype is paralleled by decreased enzymatic activities and altered physicochemical properties of HDL , thereby suggesting that the intrinsic properties of HDL particles, rather than low HDL-C levels per se, are determinants of antioxidative deficiency. In each study population (MetS, type 2 diabetes, and normotriglyceridemic low HDL-C phenotype), HDL particles enrichment in TG and CE depleted, potentially reflect elevated CETP activity and/or reduced HL activity; these alterations correlated with the diminished antioxidative activity (75,76). Mechanistically, the relationship between TG enrichment of HDL particles and impairment of antioxidative activity can be explained by the fact that the replacement of CE by TG in the HDL lipid core considerably alters the conformation of the central and C-terminal domains of apoA-I, which are critical for HDL to act as an acceptor of oxidized lipids. Moreover, replacement of CE by TG in spherical HDL decreases the conformational stability of apoA-I, resulting in TG-containing particles, which are unstable and which lose apoA-I upon storage (77).

Replacement of apoA-I by acute-phase proteins, primarily SAA, in small, dense HDL particles under conditions of chronic inflammation may represent another mechanism contributing to the impairment of HDL antioxidative activity. As in the case of the replacement of CE by TG, the replacement of apoA-I by SAA may cause deficient activity of HDL as an acceptor of oxidized PL, resulting in their elevated accumulation in LDL (36).

Altered enzymatic activities also contribute to the antioxidative deficiency of small, dense HDL. PAF-AH and PON1 activities are consistently lower in all HDL subfractions from patients with type 2 diabetes compared with matched normolipidemic control subjects . Moreover, PAF-AH and PON1 activities positively correlate withHDL antioxidative activity,suggesting that these enzymes are implicated in the deficiency of HDL anti-oxidative function (75). In type 1 diabetes, serum concentrations of PON1 are reduced to such an extent that diminished oxidative protection of LDL by HDL in vitro results (58). Consistent with this mechanism, inactivation of HDL-associated enzymes, such as PON1 or LCAT, by oxidation and/or glycation leads to decreased capacity of HDL to protect LDL from oxidative stress (56). The role of enzymes in HDL antioxidative deficiency is also consistent with data obtained in obese leptin-deficient (*ob/ob*), LDL-R$^{-/-}$ mice .

An antioxidative deficiency of HDL may also be observed when antioxidative activity is measured in total HDL, rather than in individual HDL subfractions. Total HDL from humans and rabbits lose the ability to protect LDL against oxidation by artery wall cells in coculture during induction of the acute phase, concomitant with decreases in PON1 and PAF-AH activities (77). Total HDL from mice that are genetically predisposed to diet-induced atherosclerosis do not protect LDL against oxidation in cocultures of artery wall cells when the mice are fed an atherogenic diet, injected with

LDL-derived oxidized PL, or infected with influenza A virus. Such loss of antioxidative activity of murine HDL is accompanied by decrease in PON1 activity. In addition, antioxidative deficiency of total HDL is observed in both apoE$^{-/-}$ and apoA-II transgenic mice (36,37).

Furthermore, total HDL-mediated protection of LDL from oxidation by Cu^{2+} is compromised in postmenopausal compared with premenopausal women. This effect is paralleled by decreased plasma levels of HDL-C, elevated HDL levels of TG, and increased HDL oxidability in the postmenopausal group; the two latter parameters are significantly correlated (78). By contrast, serum PON1 activity did not differ between the groups, lending further support to the hypothesis that alterations of HDL core lipid composition are a key determinant of the antioxidative function of HDL particles (79). Finally, a diminished capacity of HDL to remove lipid hydroperoxides from erythrocyte membranes and attenuated HDL PON1 activity are features of poorly equilibrated type 1 diabetes (38).

Despite the fact that HDL antioxidative deficiency has been extensively documented in atherogenic dyslipidemias using in vitro assays, direct evidence for its presence in vivo is still lacking.

4.6.3 Anti-Inflammatory Activity

HDL particles, possessing antioxidative activity, within the normal range can prevent formation of or inactivate proinflammatory oxidized PL produced during LDL oxidation and are therefore anti-inflammatory. Such potent anti-inflammatory activity becomes deficient and even transforms into in vitro pro-inflammatory action under conditions favoring development of atherosclerosis. In contrast with functional HDL, proinflammatory dysfunctional HDL is unable to protect LDL from oxidation by arterial wall cells and to prevent monocyte migration induced by oxLDL (29). Total HDL from CHD patients with normal or elevated HDL-C levels are proinflammatory in both cell culture and cell-free fluorescent assays. Similarly, HDL from mice that are genetically predisposed to diet-induced atherosclerosis become proinflammatory when the mice are fed an atherogenic diet, injected with LDL-derived oxidized PL or infected with influenza A virus (29,36).

Formation of proinflammatory HDL correlates with decreases in the activities of various HDL-associated enzymes, such as PON1, PAF-AH, and LCAT, which are replaced by acute-phase proteins, such as SAA and ceruloplasmin; indeed, the content of these proteins in HDL increases during an inflammatory response.

Interestingly, HDL PON1 activity is not decreased in CHD patients possessing proinflammatory HDL, indicating that factors other than PON1 determine anti-inflammatory HDL dysfunction in

vitro (80). In this study, plasma TG levels were markedly elevated (+76%) in CHD patients compared with control subjects (80), suggesting that concomitant HDL enrichment in TG might have significantly contributed to the formation of dysfunctional HDL as proposed elsewhere (75,76).

Intriguingly, all of the alterations in HDL composition that lead to attenuated anti-inflammatory and anti-oxidative activities (depletion in CE, apoA-I, PON1, and LCAT and increase in TG and SAA) are observed during inflammation and in the acute-phase response (80). The pro-inflammatory and prooxidative rearrangement of HDL particles and formation of LDL-derived oxidized PL have been hypothesized to form part of an evolutionary conserved mechanism of nonspecific innate immunity aimed to protect against infection (37). Such an innate inflammatory response may include subnormal levels of HDL-C, increased HDL-TG content, and altered HDL apolipoprotein composition, all of which impair cholesterol efflux capacity as well as the antioxidant and anti-inflammatory activities of HDL particles. These modifications in HDL may be aimed to redirect cholesterol from the liver to immune cells, particularly macrophages, during infection. Such a response to acute infection or injury can be advantageous in the short term but may become maladaptive in the long term. A sustained response that is not able to repair the injury, such as an emerging atherosclerotic plaque, which can be considered as a local inflammation , may lead to a chronic alteration in plasma lipid levels; such a response may become harmful, accelerating the formation of atherosclerotic lesions (81). This mechanism is consistent with a recent hypothesis that accelerated development of atherosclerosis in old age is related to increased inflammation and concomitant endothelial dysfunction during early life. Within this concept, classic lipid changes associated with MetS (low HDL-C and elevated TG levels) are envisioned as a highly conserved evolutionary response aimed to repair tissue (81).

The specific association of the deficient antioxidative activity with small, dense together with the direct mechanistic link between anti-inflammatory and antioxidative activities suggests that small, dense HDL is a major subset of the total HDL particle population, which is responsible for both potent anti-inflammatory activity under normal conditions and for deficient activity under pro-atherogenic, pro-inflammatory conditions (76,29,75).

4.7 Physiological Relevance of Defective High-Density Lipoprotein Function in Dyslipidemia and Metabolic Disease

The attenuated atheroprotective properties of HDL in metabolic disease raise the possibility of an indirect putative proatherogenic effect of these particles. Indeed, attenuated cholesterol efflux capacity of HDL can result in enhanced accumulation of cholesterol in the arterial wall and reduced RCT flux. Reduced efficiency of cholesterol flux through the RCT pathway is thought to account for the epidemiological link between subnormal HDL-C levels and increased incidence of CV disease (29,49,47). Impaired RCT has been shown to lead to accelerated atherosclerosis in subjects with Tangier disease and in some cases of LCAT deficiency (77). However, no data are available to our knowledge on the direct link between atherogenesis and the cholesterol efflux capacity of HDL particles, although infusion of apoA-I Milano/phospholipid complex has been shown to lead to a reduction in atheroma volume in patients with acute coronary syndromes, suggestive of plaque cholesterol efflux (73).

A deficiency in the antioxidative and anti-inflammatory properties of HDL may also result in accelerated atherosclerosis. The oxidation hypothesis of atherosclerosis postulates that oxidation of lipoproteins, primarily LDL, in the arterial wall is a key element in atherogenesis. The validity of this statement has been confirmed in innumerable. Its important corollary is that deficient LDL protection from oxidation may accelerate atherogenesis. Recent data indicate clearly that impairment of the antioxidative activity of small, dense HDL in dyslipidemias involving low HDL-C levels is intimately associated with elevated oxidative stress, a newly recognized CV risk factor , and may therefore contribute to enhanced atherogenesis (82). Indeed, dyslipidemic subjects presenting with atherogenic low HDL-C levels (MetS, type 2 diabetes, and a normotriglyceridemic low HDL-C phenotype) are characterized by both deficient antioxidative activity of small, dense HDL and elevated systemic oxidative stress assessed as plasma levels of 8-isoprostanes, products of nonenzymatic oxidation of arachidonic acid (75,82,83). Furthermore, HDL antioxidative activity and plasma 8-isoprostanes are negatively correlated. In addition, in subjects with controlled type 2 diabetes, plasma 8-isoprostanes negatively correlate with HDL-C levels, whereas in subjects with a normotriglyceridemic low HDL-C phenotype, plasma 8-isoprostanes positively correlate with an elevated ratio of total cholesterol/HDL-C, thereby reflecting an excess of atherogenic nonHDL-C relative to antiatherogenic HDL-C levels (75,76). The elevation of plasma 8-isoprostanes in subjects with low HDL-C dyslipidemias is consistent with the elevation of F2α-isoprostanes in apoA-I deficient mice, emphasizing the link between oxidative stress and HDL deficiency. Mechanistically, HDL enrichment in TG may play a role in both elevated oxidative stress and the deficiency in HDL

antioxidative activity, as suggested by strong association between plasma levels of oxLDL and the TG/HDL-C molar ratio in elderly subjects (77).

The presence of defective HDL can facilitate or even trigger accumulation of LDL-derived proinflammatory oxidized PL in vivo, resulting in compromised anti-inflammatory activity (80). Functional small, dense HDL particles may in turn provide protection of LDL against oxidative stress in the subendothelial space of the arterial wall via removal of oxidized lipids from LDL, with inactivation and subsequent transfer to the liver mediated by SR-BI. This mechanism may account, at least in part, for the negative results of a large-scale placebo-controlled trials that did not show any beneficial effect of low-molecular-weight antioxidants, primarily vitamin E, on the development of CV disease. The Nutrition Committee of the American Heart Association Council on Nutrition, Physical Activity and Metabolism has recently concluded that "the existing scientific database does not justify routine use of antioxidant supplements for the prevention and treatment of CV disease". Moreover, a meta-analysis of performed trials suggests that supplementation with vitamin E may even increase all-cause mortality. Some authors interpreted these data to indicate that low-molecular-weight antioxidants do not play a key role in the protection of LDL from oxidation in vivo; by contrast, small, dense HDL may constitute a central element of such protection (79).

The impaired antioxidative activity of small, dense HDL particles in atherogenic dyslipidemia is intimately linked to the presence of a constellation of CV risk factors, including hypertriglyceridemia, hyperglycemia, hyperinsulinemia, insulin resistance, and a disequilibrium between circulating levels of atherogenic apoB-containing lipoproteins and antiatherogenic HDL in favor of the former. All of these factors are independently characterized by their significant association with elevated systemic oxidative stress (75,76). Such correlational data strongly suggest that HDL particles function as a biosensor of oxidative stress, integrating a wide spectrum of prooxidant signals; the integration of such signals is in turn expressed as attenuated HDL antioxidative activity. Diagnostic detection of HDL possessing deficient antioxidative activity may therefore serve as a novel biomarker to assess elevated CV risk.

THE AIM OF THE STUDY

The pathological evolution of obesity to the development a condition of metabolic syndrome enhances the alterations of glucose and lipid metabolism. According to a molecular point of view, the cluster of metabolic consequences, in overweight and complicated overweight, present a condition of elevated oxidative stress and a low grade inflammation. The inflammatory response promotes the formation of free radicals and reactive oxygen species and the resultant consumption of antioxidant molecules. Similarly oxidative stress stimulates the production of inflammatory cytokines while maintaining or even extending the same pathogenic mechanisms. At the level of the systemic circulation, reactive oxygen species can induce the peroxidative degradation of apoproteins and lipids that constitute the plasma lipoproteins. The aim of the study was to observe structural and functional modification of plasma lipoproteins in obese subjects with or without metabolic syndrome. We proposed to evaluate the lipoproteins change in composition, activity and susceptibility to peroxidation.

Through the use of appropriate fluorescent probes we investigated the susceptibility to peroxidation of the different classes of lipoproteins, differentiating between the lipoproteins core and evelope comportment.

MATERIALS AND METHODS

5.1 Subjects

The recruitment took place at Istituti Clinici di Perfezionamento, Milano.

To be included in the study, patients had to have the following characteristics: age between 18 and 55 years (but not in menopause in women), BMI between 25 and 35, absence of major diseases, lack of drug therapies in place able to influence the lipid profile (eg. progestogens, statins, oral hypoglycemic agents, treatment with thyroid hormone synthesis), lack of use of antioxidant supplements, smoking less than 5 cigarettes per day and consumption of alcohol less than 25 g / day. All subjects signed an informed consent for membership in the study. The inclusion criteria are summarized in Table 2.

Table 2. Inclusion criteria.

Criteria
Female and male
Aged between 18 and 55 years (women: 55 years if not yet in menopause or pre-menopause)
BMI between 25 and 35 Kg/m^2
Lack of hypo-glycemic treatments or drugs that alter the lipoprotein metabolism in the last 6 weeks
Lack of use of antioxidant supplements
Alcohol consumption less than 25 g/day
Smoking less than 5 cigarettes
Absence of hormone treatments (including estrogen-progestin)
Absence of major diseases (heart failure, liver disease, kidney disease, cancer, etc.).
Provide a written informed consent

The recruited subjects underwent a dietary first visit. Family history information was collected for overweight and obesity, dyslipidemia, diabetes and cardiovascular disease, and later was conducted physiological history, investigating in depth the level of physical activity, the characteristics of the hive and urine output, the presence of cigarette smoking or consumption of alcoholic beverages, the presence of known allergies to drugs, food or pollen and the characteristics of the menstrual cycle or the possible presence of menopause in women, were finally carried the proximate and remote medical history, investigating the presence of any major diseases or the presence of ongoing drug therapy. The patient was then submitted to a complete physical examination, with measurement of blood pressure.

After the visit, it was conducted an interview on the history food eating habits of the patient. Dietary food recall was collected with a recall of 24 h plus a survey of food frequency consumption.

Then patients recruited were invited to the clinic the day following the first visit, after an overnight fast, and to undergo a venous blood sample (20 ml) collected in heparinized tube. The blood sample was sent to the Laboratory of Biochemistry, University of Milan (LITA Vialba). From blood samples, plasma was obtained by centrifugation at 1000xg at 4 ° C. The plasma was stored at -80 ° C until use. The plasma was analyzed to evaluate the oxidative balance and plasma susceptibility to peroxidation of plasma lipoproteins.

Immediately after blood collection, patients underwent a complete anthropometric measurements: weight, height, BMI, waist circumference, hip circumference, calculation of waist to hip ratio, arm circumference, wrist circumference, biceps skinfold, triceps skinfold, subscapular skinfold. In addition, patients were required to undergo in the days immediately following, a venous blood sample to perform some routine blood analysis: CBC formula, total cholesterol, HDL cholesterol, triglycerides, blood glucose, insulin, glycated hemoglobin , APOA1, ApoB, creatinine, AST, ALT, CRP, serum iron, TSH, uric acid. The complete list of evaluations carried out are summarized in Table2. The enrolled patients were divided into two study groups: group A consisting of patients with metabolic syndrome according to the criteria of the ATP-third (Table 3) and group B consists of patients without metabolic syndrome.

Table 3.

General medical history (family, physiological, pathological proximate and remote)
Physical examination with measurement of PA
Nutritional anamnesis
Anthropometric measurements (weight, height, BMI, skinfolds and circumferences)
Routine blood tests (CBC with formula, total cholesterol, HDL cholesterol, triglycerides, blood glucose, insulin, glycated hemoglobin, APOA1, ApoB, creatinine, AST, ALT, CRP, serum iron, TSH, uric acid)

5.2 Blood collections and separation

A fasting blood sample was drawn for each subject enrolled in the study. The sample was collected in appropriate tubes of 4 mL heparinized (1 ml of sodium heparin / mL of blood) and stored at 4 ° C to be transported in our laboratories where it is provided for the separation of plasma. Each stage of the separation was carried out keeping the sample at a temperature of about 4 ° C by using a bath of water and ice. The tubes were centrifuged at 2500 rpm for 10 minutes at 4 ° C (centrifuge model ALC. Pk121R). After the first spin the supernatant (plasma) was collected and transferred to another tube.(With a plastic pasteur has been removed the whitish pellets (consisting of lymphocytes and platelets) that separates the underlying eritociti. The eritociti underwent two cycles of washing with an equal volume of a solution of EDTA / NaCl 1:3 and centrifuge at 3000 rpm for 10 min at 4 ° C). The plasma was subjected to further centrifugation at 3000 rpm for 10 min at 4 ° C before being aliquoted and stored at -80 ° C until use (84).

5.3 Kinetics of plasma peroxidation

Plasma peroxidation was induced at 37 °C by adding 120 µL of $CuSO_4$ 1 mM to 120 µL of plasma flow-volume of 2200 µL with 10 mM PBS pH 8.0.

The peroxidation kinetics were monitored following the formation of fluorescent adducts originating from the reaction of polyfunctional aldehydes (derived from plasma lipid peroxidation)

with amino groups of plasma proteins and/or phospholipids . The development of fluorescence emission was monitored at 430 nm, setting the excitation at 360 nm, every 15 min for 8 hours.

The kinetics are expressed by a sigmoidal curve that can be divided into an initial latency phase followed by a second propagation phase. The kinetic profile of these curves allows the evaluation of at least two indices and offers a good description of the process of plasma peroxidation (fig.4). These indices are: the maximal rate of oxidation, which can be calculated from the slope of the fluorescence curve during the propagation phase, and the lag-time, expressed in minutes and calculated from the intersection of the linear regression of the propagation phase tangent with that of the lag-phase (84). All the measurements were carried out in a Jasco spectrophotofluorimeter FP-777 equipped with a cuvette holder. The temperature maintained by a Haake GD3 thermostatic circulating bath, was monitored with a Subline PT 100 digital thermometer. Salts were purchased from Sigma-Aldrich (Milan, Italy)

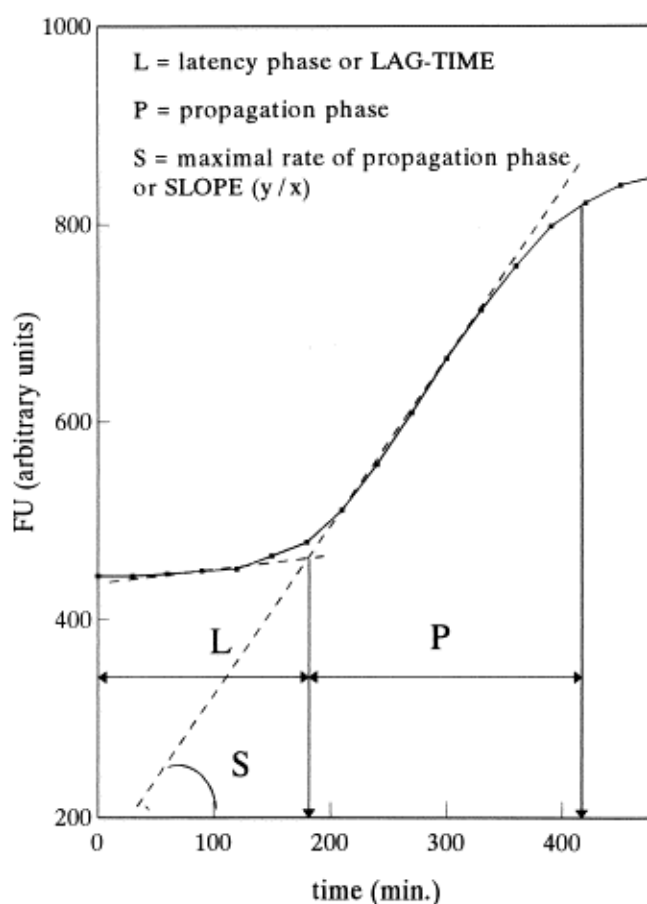Figure 4 — Fluorescence-determined kinetics of plasma peroxidation. The kinetic curve individuates three phases: a latency phase, a propagation phase, and a termination phase. From the data of fluorescence kinetics of plasmatic peroxidation it is possible to determine the time of latency (lag-time, expressed in minutes) and the maximal rate of the propagation phase (slope, expressed in arbitrary fluorescence units/min).

5.4 Plasma lipoproteins separation

Lipoproteins were separated according to their density by ultracentrifugation from a sample volume of 0.9 mL. The plasma density d = 1.006 g/mL was increased to d = 1.3 g/mL by adding an aliquot of salt KBr (Sigma) to facilitate separation.

Two solutions of KBr at density d = 1.0006 g/mL and d = 1.21 g/mL were stratifcated on the sample and we proceeded with a first centrifuge (Beckman TL100 ultracentrifuge) at 100,000 rpm T= 4 ° C (AC = 6 , DEC = 6) for 1 hour. The different fractions of lipoproteins were separated into bands clearly visible at different density: VLDL at the top, the LDL in the middle layer and just below the HDL plasma proteins remain in the lower part of the tube. The lipoprotein fractions VLDL, layered in a volume of 0.5 mL were removed from the upper layer using a fine tip pasteur by trying to minimize the disturbance on the gradient. We proceeded in taking below the LDL equal to a volume of 0.7 ml. An additional centrifuge was used for HDL . A volume of 1 ml was collected and transferred into another tube, the density adjusted with KBr and after statification with a salt solution at the density of 1.21 g / mL, was carried out a further centrifuge at 100,000 rpm T = 4 ° C (AC = 6, DEC = 6) for 2 hours. The HDL fraction of lipoproteins, layered in a volume of 1 ml were removed from the top layer. The three lipoprotein fractions brought to a volume of 1 ml with PBS 10 mM NaCl 154 mM pH 7.4 were dialyzed to remove excess salt, overnight against 10 mM NaCl 154 mM PBS pH 7.4, buffer lipoprotein ratio = 1:100 at 4 ° C. Dialysis tubing size 1 Inf. Dia 8/32'' MWCO 12-14000 Daltons were used (Medicell International Ltd) The next morning, lipoproteins were removed by dialysis and stored at -80 ° C. All fluorescence measurements were carried out in a Jasco spectrophotofluorimeter FP-777 equipped with a cuvette holder. The temperature maintained by a Haake GD3 thermostatic circulating bath, was monitored with a Subline PT 100 digital thermometer (85). Salts were purchased from Merk.

5.5 Lipoprotein characterization

The protein concentration of each lipoprotein fraction was determined with the Lowry method (86) using bovine serum albumin as standard. Total lipids were extracted from each lipoprotein fraction following the Folch procedure (85). The phospholipid content was determined according to Bartlett (87); cholesterol was determined using a reagent kit from Scalvo (85). All reagents were purchased from Sigma-Adrich (Milan-Italy).

5.6 Labelling of lipoproteins with fluorescenct probes

A lipoprotein separation was carried out from plasma previously incubated for one hour at room temperature with fluorescent probes. To labell the hydrophobic core of LDL and HDL, plasma was incubated with cholesteryl pyrenyl hexanoate, final concentration of 10 nmol/mL (Sigma C-2205 PM=685; P_6CHOL) mixed with egg phosphatidylcholine (1:10 mol:mol) (EPG). Decanoyil phosphatidylcholine 5 nmol/ml (Sigma P-7657 PM=850,1; $P_{10}PC$) was used to probes the lipoproteins envelope. The probes were suspended with chloroform methanol 2:1, thens evaporated with nitrogen. The probes were dissolved with a phisiological solution and then added to plasma. (85).

5.7 Lipoprotein peroxidation

The two different pyrene derivatives, pyrene decanoyil phosphatidylcholine ($P_{10}PC$) and cholesteryl pyrenyl hexanoate (P6Chol), were used to follow lipid peroxidation in low and high density lipoproteins. We used the fluorescence decrease of P6Chol to monitor the lipid peroxidation in the hydrophobic core of LDL and HDL, and that of the amphipatic probes $P_{10}PC$, to follow lipid peroxidation in the envelope of both lipoproteins.

Peroxidation of LDL and HDL was induced by incubating lipoproteins (0.1 mg protein/ml) suspended in 10 mM Tris-HC1, 154 mM NaC1 (pH 7.4) at 37°C with 2,2'-azobis-2-metil-propanimidamide (AAPH). The AAPH is a water soluble compound which decomposes at 37 ° C generating a continuous flow of free radicals.

The oxidation of labelled LDL and HDL was monitored by the decrease in the pyrene's fluorescence emission intensity at 379 nm (excitation 343 nm) . This decrease was expressed as $((Fo-Ft)/F o) \times 100$, where F o represents the fluorescence intensity at zero time of peroxidation and F t the residual fluorescence for a given peroxidation time.

The fluorimetrically determined peroxidation profile of each lipoprotein sample can be divided into latency, propagation and steady state phases and allows the evaluation of lag time, expressed in minutes, calculated as the intercept of the linear least square regression of the propagation phase with that of the latency phase (85). All reagents were purchased from Merk.

5.8 Paraoxonase

Plasma PON1 activity was assayed using two synthetic substrates: paraoxon (diethyl-*p*-nitrophenyl phosphate) and phenyl acetate.

PON1 activity toward paraoxon was determined by measuring spectophotometrically at 405 nm the initial rate of substrate hydrolysis to *p*-nitrophenol. The absorbance was monitored in an assay mixture containing 10 µl serum and 10 mM paraoxon-ethyl (Sigma-Aldrich, Mialn, Italy). in a Tris–HCl buffer CaCl2 1 mM (pH 8.0). The measurements were carried out at 37°C with VICTOR3™ multilabel readers PerkinElmer equipped with microplate reader. The blank sample containing buffer mixture with plasma was run simultaneously. The enzyme activity was calculated from molar extinction coefficient of *p*-nitrophenol ($18,290 M^{-1}$ cm^{-1}) and was expressed in Uml^{-1}; 1U of enzyme hydrolyses corresponds to 1 nmol of paraoxon per min. Salts were purchased from Merk.

Enzyme activity toward phenyl acetate (arylesterase activity) was determined by measuring the initial rate of substrate hydrolysis in the assay mixture containing 5 ul serum, 10 mM phenyl acetate (Sigma-Aldrich, Milan, Italy) in a Tris–HCl buffer CaCl2 1 mM (pH 8.0). The absorbance was monitored for 1 min at 270 nm and the activity was calculated from molar extinction coefficient (1310 M^{-1} cm^{-1}). The results are expressed in Uml^{-1}; 1U of arylesterase hydrolyses corresponds to 1 mol of phenyl acetate per min (88).

The measurements were carried out at 37°C with V-550 Jasco Spectrophotometer. Salts were purchased from Merk.

5.9 Plasma ROS levels

Plasma ROS levels were measured using a colorimetric kit (d-ROM test; Diacron, Grosseto, Italy); the results are expressed as H2O2 equivalents, according to the manufacturer's instructions (89).

5.10 Statistics

The average values in the SM and OB groups were compared with each other and compared to those previously obtained by a group of normal-weight subjects, matched for age and sex. The differences between the two groups were analyzed statistically using the Mann-Whitney test and were considered significant for p values ≤ 0.05.

RESULTS AND DISCUSSION

6.1 Patients

To date 98 patients were enrolled of which 70 obese (40 males and 30 females) and 28 obese (20 males and 8 females) with metabolic syndrome. As the number of female subjects with metabolic syndrome is currently few in number and gender influence the lipoprotein metabolism, we present only the results for males.

Therefore, the following results refer to the comparison between the group of 40 obese men without the syndrome (OB) and 20 obese men with metabolic syndrome (MS) (Tab. 4.). the two groups are homogeneous for age and BMI. Significant are the differences in some parameters that characterize the metabolic syndrome such as triglycerides and HDL. The waist circumferences are not diffent in the two groups but there is a significant difference in WHR. WHR expresses the ratio between waist circumference and hip circumference. This index is used in the medical field to assess the distribution of body fat. No differences were found in blood glucose levels while insulin and HOMA, two parameters involved in glucose metabolism are significative different. The two groups differ for PCR, a protein which increases its blood concentration in inflammatory processes. Arilesterase activity of enzyme paraoxonase was very similar in both groups, while paraoxon activity was reduced by 15% in MS than in OB, but this reduction was not statistically significant.

Tab. 4

	OB (n=40)		SM (n= 20)		p
	means	SEM	means	SEM	
AGE (YEARS)	44	1.8	48	2.3	ns
BMI (kg/m^2	30.1	0.4	30.9	0.4	ns
Skinfold triceps (mm)	21.8	1.1	24.3	1.6	ns
Skinfold biceps (mm)	16.3	0.9	15.2	1.3	ns
Skinfold subscapolar(mm)	33.9	2.0	35.2	0.9	ns

WHR	**0.97**	0.008	**1.02**	0.05	**<0.01**
Waist circumferences(cm)	**105.0**	1.2	**107.5**	0.9	**ns**
Triglycerides (mg/dl)	**111**	9	**170**	23	**<0.05**
Total Cholesterol (mg/dl)	**205**	5.7	**222**	7	**ns**
HDL (mg/dl)	**52**	1.7	**42**	1.8	**<0.01**
DBP (mm Hg)	**82**	1.5	**80**	1.4	**ns**
SBP(mm Hg)	**132**	1.8	**135**	2.6	**ns**
gluc (mg/dl)	**91**	1.3	**97**	2.9	**ns**
Insulin (μU/ml)	**7.0**	0.7	**13.1**	1.1	**<0.01**
HOMA	**1.5**	0.15	**3.2**	0.3	**<0.01**
CRP (mg/dl)	**0.17**	0.01	**0.44**	0.06	**<0.01**
Paroxon activity(U/ml)	**127**	13	**107**	15	**ns**
Arilesterase activity (U/ml)	**74**	2.5	**72**	3.4	**ns**

6.2 Susceptibility to peroxidation and ROS plasma levels

The parameters indicative of the susceptibility to peroxidation (lag-time and slope of the kinetics of peroxidation) and ROS plasma levels were significantly worse in the group of obese (OB) and in the obese with metabolic syndrome (MS) than in healthy controls CT , but there were no significant differences between OB and MS (Tab.5).

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Tab.5

	CT (n =50)		OB (n=40)		SM (n= 20)	
	means	SEM	means	SEM	means	SEM
Lag-time (min)	167*	2.5	147	3.2	145	4.0
Slope (% decrease/min)	1.05*	0.03	1. 3	0.06	1.3	0.09
ROS (mg/dl)	22.4*	07	30.4	0.6	29.6	0.9

* p< 0.05 vs OB e SM

6.3 Lipoproteins composition

Lipoproteins analysis showed statistically significant differences between the two groups in lipid composition. HDL and LDL in the MS group had a higher content of triglycerides (tab.6) according with data present in literature (62).

Tab.6

	VLDL		LDL		HDL	
	OB (n=40)	SM (n=20)	OB (n=40)	SM (n=20)	OB (n=40)	SM (n=20)
Proteins (mg/ml)	258 ±23	327 ±41	930 ± 50	1042 ±64	763 ±38	832 ± 81
Phospholipids (% w/w)	19.5 ± 1.5	15.3± 2.4	34.6 ± 0.8	32.5± 0.8	46.0 ± 2	42.0 ± 3
Cholesterol (% w/w)	29.2 ± 1	30.7 ±0.8	48.9 ± 0.8	47.2 ±1.0	37.3 1.2	39.6 ±2
Triglycerides (% w/w)	51.3 ±1.6	54.0 ±2.7	16.4±0.7*	21.2 ±1.4	16.7 ± 1*	26.1 ± 1

* P < 0.05 OB vs SM

6.4 HDL susceptibility to oxidation

The hydrophobic core of HDL in subjects OB has a greater resistance to peroxidation (lag-time).The other parameters indicative of susceptibility to peroxidation of lipoproteins HDL and LDL despite being the best in the group OB, statistically did not differ significantly between the two groups (tab.7).

Tab. 7

	OB		SM		p
	means	SEM	means	SEM	
Hydrophilic Envelope:					
Lag-time (min)	14.9	0.9	11.3	1	ns
Slope (% decrease/min)	1.45	0.1	1.65	0.09	ns
Hydrophobic Core :					
Lag-time (min)	9.6	0.5	4.3	0.8	0.03
Slope (% decrease/min)	1.26	0.3	1.51	0.05	ns

CONCLUSION

The results of this study revealed that overweight and obesity may increase the risk of peroxidation of HDL and LDL. The metabolic syndrome can further accentuate the degradation processes, especially in the hydrophobic core of HDL.

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