Minireviews

A Compendium of the Biological Effects of Apolipoprotein A-I_{Milano}

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ABSTRACT

Obesity is a pathologic condition generated by an energy imbalance, that is, excess caloric consumption, leading to weight gain and metabolic disturbances characterized by adipose tissue inflammation and hyperglycemic conditions. In line with these observations, increasing evidence causally links inflammation, or the molecules and networks integral to inflammatory response, to the development of obesity and the complications that emerge from this pathology, such as cardiovascular, neurologic, respiratory, and metabolic illnesses, as well as sepsis and cancer. Not surprisingly, this chronic and abnormal metabolic background leads to constant derangements in innate and adaptive immunity. It is well known that high-density lipoprotein (HDL) possesses anti-inflammatory and antioxidant properties, and various studies have highlighted an emerging role of HDL in modulating immune function. The efficacy of synthetic HDL (sHDL) containing the recombinant form of apoA-I_{Milano} (sHDL-apoA-I_M), originating from the observation that carriers of this mutation have low levels of HDL cholesterol without increased atherosclerosis, has been largely proved in diverse animal models of atherosclerosis; however, the therapeutic use of sHDL-apoA-I_M still needs clinical validation.

One of the main limitations to the use of recombinant proteins in clinical studies lies in the unsustainable purification costs. Unpurified rice-milk-apoA-I_M demonstrated anti-inflammatory and antiatherogenic properties in a mouse model, even though administered by an unconventional way: by oral gavage. Additionally, recent data have uncovered new therapeutic applications for this sHDL-apoA-I_M. This review provides an overview of all potential application of sHDL-apoA-I_M in some inflammatory-based diseases.

SIGNIFICANCE STATEMENT

A recent study demonstrated that oral administration of rice-seed protein extracts containing the apoA-I_M (i.e., the milk-apoA-I_M) reduced atherosclerosis development in a mouse model. Moreover, the rice-milk-apoA-I_M preserved both in vitro and in vivo anti-inflammatory properties, as observed when sHDL-apoA-I_M was given by intravascular infusion. Besides, various studies suggested that sHDL-apoA-I_M could positively affect other inflammatory-based diseases. Together, these data might represent a new starting point for “sHDL-apoA-I_M-based therapies” in chronic degenerative disease.

Introduction

Obesity is currently considered a worldwide health problem because of its high prevalence (i.e., nowadays more people are obese than underweight) and its association with endothelial dysfunction, dyslipidemia, insulin resistance, and chronic low-grade inflammation (Frydrych et al., 2018). Obesity is a chronic condition caused by an energy imbalance (i.e., excess food intake), leading to weight gain and metabolic changes characterized by tissue stress and organ dysfunction (Frydrych et al., 2018). Moreover, the high incidence of obesity

in early youth causes an extended exposure to adipose tissue inflammation and hyperglycemic conditions. In line with these observations, considerable evidence causally links inflammation, or the molecules and networks integral to inflammatory response, to the development of obesity and the complications that emerge from this pathology (i.e., cardiovascular, neurologic, respiratory, and metabolic illnesses, as well as sepsis and cancer) (Fig. 1) (Hotamisligil, 2006; Back et al., 2019; Parolini, 2019b). Specifically, chronic inflammation is first promoted by metabolic surplus, mainly localized in specialized metabolic tissues (i.e., white adipose tissue) that leads to activation of metabolic signaling pathways, including c-Jun N-terminal kinase, nuclear factor- κ B (NF- κ B), inflammasome, and protein kinase R, similar to those involved in the

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ABBREVIATIONS: AD, Alzheimer disease; apoA-I_M, apolipoprotein A-I_{Milano}; CVD, cardiovascular disease; ETC-216, sHDL constituted by recombinant apoA-I_{Milano} and 1-palmitoyl-2-oleoyl phosphatidylcholine complexes; HDL, high-density lipoprotein; IL, interleukin; IVUS, intravascular ultrasound; LDL, low-density lipoproteins; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; NF- κ B, nuclear factor- κ B; sHDL-apoA-I_M, synthetic HDL containing recombinant form of apoA-I_M; sPLA2, secretory phospholipase A2; β 2 TLR, toll-like-receptor; TNF- α , tumor necrosis factor- α ; Tregs, T regulatory cell.

Fig. 1. Schematic representation of the link between energy imbalance (i.e., excess caloric consumption, chronic inflammation, obesity and human disease).

classic inflammatory milieu (Ertunc and Hotamisligil, 2016). Not surprisingly, the clinical manifestations of obesity are central adiposity, elevated blood pressure and glucose levels, hypertriglyceridemia, and low- plasma high-density lipoprotein (HDL)-cholesterol, which is generally associated with an increased risk of premature cardiovascular disease (CVD) (Parolini et al., 2019).

High-Density Lipoprotein

The strong inverse relationship between HDL cholesterol and CVD was first established by the Framingham Heart Study (Kannel et al., 1964); however, mutations in several human genes influence HDL cholesterol levels without changing cardiovascular risk, contrary to what was expected from epidemiologic studies (Voight et al., 2012). HDL, the smallest plasma lipoprotein, has a diameter of 8–14 nm and the highest protein-to-lipid ratio, resulting in high density. It is constituted by lipids (e.g., phospholipids, cholesteryl esters, triglycerides, free cholesterol, and sphingolipids), apolipoproteins (apo) (e.g., apoA-I, apoA-II, apoC, apoE, apoD, apoM, apoA-IV, and apoA-V), lipid-transfer proteins (i.e., cholesterol ester transfer protein), and phospholipid transfer proteins, enzymes (i.e., lecithin cholesterol acyltransferase and para-oxonase1), and inflammatory-phase response proteins (e.g., serum amyloid A and apoJ). ApoA-I and apoA-II are the most abundant and important proteins, even though, as already stated, each protein present in the HDL structure has a clear biologic function. For instance, PON1 is involved in the removal of carcinogenic free radicals and in the scavenging mechanisms to ensure oxidative balance. The most established mechanistic hypothesis underlying the inverse relationship between HDL cholesterol and atherosclerosis is the ability of HDL to stimulate the reverse cholesterol transport, the process by which excess cholesterol is removed from the peripheral tissues, including the arterial wall, and transported to the liver for biliary excretion. Specifically, HDL promotes the efflux of cholesterol from peripheral cells, the first step in reverse cholesterol transport (Chiesa et al., 1998; Franceschini et al., 1999; Parolini et al., 2012a), and, recently, it has been associated with the ability of HDL to modulate

T-cell activation (Sorci-Thomas and Thomas, 2012). Indeed, HDL, by promoting cholesterol efflux from T cells, increases the fraction of Tregs (a T-cell subtype that inhibits proinflammatory T cells) and modulates inflammation (Sorci-Thomas and Thomas, 2012; Parolini, 2019b). Moreover, the cholesterol efflux capacity has been strongly and inversely associated with the incidence of ischemic events and is significantly lower in patients with atherosclerosis or CVD compared with that measured in healthy subjects (Rohatgi et al., 2014; Talbot et al., 2018). Furthermore, HDL exerts anti-inflammatory effects by modulating the cytokine-induced expression of cellular adhesion molecules, the production of proinflammatory cytokines/chemokines [including tumor necrosis factor- α (TNF- α), interleukin (IL)-1, IL-2, and IL-6, monocyte chemoattractant protein-1 (MCP-1)], and the expression of anti-inflammatory cytokines (such as transforming growth factor- β 2) in cultured endothelial cells (Barter et al., 2004; Florentin et al., 2008). Although the mechanisms responsible for these effects are not completely understood, accumulating data have shown that HDL can inhibit sphingosine kinase activity and NF- κ B translocation and can increase expression of heme-oxygenase 1 (Grunfeld and Feingold, 2008). Besides, HDL possesses antioxidant properties because it can to prevent oxidation of low-density lipoproteins (LDL) by inhibiting lipid peroxidation and by removing oxidation products, mainly lipid hydroperoxides, from the LDL (Franceschini et al., 1999; Chiesa et al., 2008; Ayala et al., 2014). Further, HDL increases the activity and stability of the endothelial nitric oxide synthase and the cyclooxygenase-2, the enzyme responsible for prostacyclin production, improving the nitric oxide and prostacyclin availability (Manzini et al., 2015). These mechanisms validate the ability of HDL to protect the endothelium from its conversion into a proaggregant and proadhesive surface by promoting smooth muscle cell relaxation and antithrombotic effects (Soma et al., 1995a; Franceschini et al., 1999; Parolini et al., 2009). Experimental data have suggested that both the SR-BI and the ATP-binding cassette, subfamily G member 1 are involved in the HDL-mediated modulation of vascular tone (Gordon et al., 2011). Finally, accumulating evidence indicates that HDL is an integral component of innate immunity, modulating immune functions that protect the host from infections (Feingold and Grunfeld, 2011; Gordon et al., 2011). Indeed, HDL, by exerting direct antimicrobial effects and by increasing the amount of Treg cells (i.e., mitigating the inflammatory response) might represent the determinant factor that positively influence the outcome of the infections (Gordon et al., 2011; Sorci-Thomas and Thomas, 2012).

Apolipoprotein A-I_{Milano}

Apolipoprotein A-I_{Milano} (apoA-I_M), the first variant of apoA-I, was discovered in 1974 in individuals from Limone sul Garda in northern Italy (Parolini et al., 2003, 2005; Marchesi et al., 2011b). This mutation is caused by a cysteine-to-arginine substitution at position 173 (R173C) (Chiesa et al., 2004). The presence of a cysteine residue results in the formation of homodimers (apoA-I_M-apoA-I_M) and heterodimers with apoA-II (apoA-I_M-apoA-II). The carriers are all heterozygous and share a lipoprotein profile characterized by very low plasma levels of HDL cholesterol with moderate hypertriglyceridemia, a condition generally associated with

a high risk of premature CVD (Soma et al., 1994, 1995a). This mutation seems to protect against the development of atherosclerosis and CVD (Chiesa et al., 2008). Indeed, compared with control subjects, apoA-I_M carriers showed no evidence of increased vascular disease at the preclinical level (Parolini et al., 2009). This clinical paradox was partly clarified by in vitro and in vivo studies demonstrating that apoA-I_M is more effective than normal apoA-I in: 1) promoting cholesterol efflux from cells, 2) protecting LDL from oxidation, 3) inducing lipolysis and weight loss, and 4) reducing markers of inflammation and plaque vulnerability (Franceschini et al., 1999; Parolini et al., 2005; Ibanez et al., 2012; Lindahl et al., 2015). Consistent with the apoA-I_M paradox are data from the Reasons for Geographical and Racial Differences in Stroke (REGARDS) study showing that HDL cholesterol levels <40 mg/dl were associated with reduced risk of incident coronary heart disease in black participants (Penson et al., 2019). Madsen et al. (2018), however, reported that, in the general population, low concentrations of HDL cholesterol were associated with a high risk of infectious disease. Together, these observations support the concept that HDL quality, rather than HDL cholesterol concentrations, might explain the actual role of HDL in chronic disease (Mackey et al., 2012).

This review collects the current data on the biologic effects of apoA-I_M, promoting the idea that synthetic HDL (sHDL) containing the recombinant form of apoA-I_M (sHDL-apoA-I_M) still represents a therapeutic option for the treatment of inflammatory-based disease.

Cardiovascular Disease

Atherosclerosis, the dominant cause of CVD, is a chronic inflammatory disease characterized by the progressive accumulation of lipids and inflammatory cells within the arterial wall that can lead to clinically significant endpoints, that is, myocardial infarction, peripheral arterial disease, and stroke (Ganzetti et al., 2019; Parolini et al., 2019). Epidemiologic, pathophysiological, genetic, and clinical evidence indicate dyslipidemia as a determinant factor involved in the development of atherosclerosis (Herrington et al., 2016). In humans and in certain genetically modified mice (Busnelli et al., 2017, 2018a), circulating levels of cholesterol in LDL are directly related to the development of atherosclerosis and subsequent CVD, whereas HDL cholesterol concentrations are inversely related (Kannel et al., 1964; Herrington et al., 2016; Manzini et al., 2019). Notably, statins are powerful cholesterol-lowering medications and have provided outstanding contributions to the prevention of CVD and to the dramatic declines in vascular mortality (up to 50% in the highest dosage) observed in high-income countries (Herrington et al., 2016; Kazi et al., 2017). Furthermore, the development of atherosclerosis has been halted or even reversed in different animal models by transgenic overexpression or exogenous administration of apoA-I (Liu et al., 1994; Chiesa et al., 2008). To date, however, it has been difficult to reduce successfully CVD risk with drugs that increase HDL cholesterol, such as fibrates, niacin, or inhibitors of cholesteryl ester transfer protein (Parolini et al., 2017; Pownall and Gotto, 2019). Lastly, a recent study demonstrated that elevated levels of HDL cholesterol are associated with increased risk of cardiovascular mortality (Madsen et al., 2017). To explain

these conflicting results, it is important to highlight that, in contrast to the proatherogenic role played by LDL cholesterol, HDL cholesterol is only a nonfunctional surrogate marker for estimating HDL particle number and size, without deciphering the heterogeneous composition, and hence the functionality, of HDL (Mackey et al., 2012). As already mentioned, HDL displays many atheroprotective functions, such as the induction of nitric oxide-mediated vasodilation, decreased thrombosis risk via platelet stabilization, and plaque stabilization via a decrease in inflammation and leukocyte adhesion (Parolini et al., 2009; Di Bartolo et al., 2018). Besides, increased plaque vulnerability has been observed in *ApoE*-deficient mice treated with anti-apoA-I autoantibodies, and this effect was mediated by toll-like receptor (TLR)-2 and TLR-4 signaling pathways without affecting atherosclerotic plaque size (Montecucco et al., 2011, 2015; Vik et al., 2013). Additionally, various studies have shown that atherosclerosis is influenced by the immune system, with cytokines involved in all stages of the disease (Back et al., 2019). The Canakinumab Antiinflammatory Thrombosis Outcomes Study (CANTOS) trial demonstrated that anti-inflammatory therapy, targeting the interleukin-1 β (IL-1 β) pathway, dramatically lowered plasma concentration of IL-6 and of C-reactive protein, reducing the rate of recurrent cardiovascular events compared with placebo, independently of lipid-level lowering (Ridker et al., 2017; Parolini, 2019b). By contrast, in the CIRT study, low-dose methotrexate did not reduce levels of IL-1 β , IL-6, or C-reactive protein and was not associated with a lower incidence of cardiovascular events (Ridker et al., 2019).

Year 2020 is the 30th birthday of the pioneering study by Badimon et al. (1990), which demonstrated that HDL could be used as a therapeutic agent for atherosclerosis regression. This first observation represented the “kickoff” for various experimental studies that used sHDL-apoA-I_M (Parolini et al., 2009; Kühnast et al., 2015). Briefly, intravenous infusions of sHDL-apoA-I_M in *ApoE*-deficient mice fed a high-cholesterol diet caused a regression of established atherosclerotic plaque, together with a reduction in both lipid and macrophage content (Shah et al., 1998). In New Zealand White rabbits, short-term administration of sHDL-apoA-I_M was efficacious in reducing atherosclerosis progression (Marchesi et al., 2008b; Parolini et al., 2008), as well as inducing rapid regression and improving plaque stability, as proved by a dramatic reduction in markers of plaque vulnerability, such as tissue factor, MCP-1, and matrix metalloproteinase-2 (Ibanez et al., 2008, 2012; Ganzetti et al., 2019). Furthermore, these studies demonstrated that the acute regression and the shift toward a stabilization of established atherosclerotic lesions were maintained up to 6 months after the end of sHDL-apoA-I_M administration (Parolini et al., 2008; Ibanez et al., 2012). Moreover, several preclinical data indicated that sHDL-apoA-I_M is effective in restenosis prevention (Soma et al., 1995a; Deller et al., 2016). In the earliest study, rabbits fed a high-cholesterol diet were injected with sHDL-apoA-I_M 5 days before balloon injury at both femoral and iliac arteries and for 5 days after surgery. At sacrifice, that is, 3 weeks later, sHDL-apoA-I_M-treated rabbits showed reduced intimal thickening compared with that measured in the control group (Ameli et al., 1994). A significant decrease in neointima formation and in smooth-muscle cell proliferation was instead detected when the same dosing schedule was applied to hypercholesterolemic

rabbits undergoing vascular injury through perivascular manipulation (Soma et al., 1993, 1995b). The efficacy of a single intramural infusion of sHDL-apoA-I_M was investigated in a porcine model of coronary stenting. Pigs were injected with sHDL-apoA-I_M at a very low concentration (i.e., 5.6 mg) before stent implantation. At the end of the experimental protocol (i.e., 4 weeks later) in sHDL-apoA-I_M-treated animals, a smaller neointima area and intima/media ratio were observed compared with those measured in placebo-treated pigs (Kaul et al., 2003). Based on these results, the impact of sHDL-apoA-I_M on myocardial ischemia was investigated using both ex vivo and in vivo models. The ex vivo study demonstrated that ETC-216 (i.e., sHDL), constituted by recombinant apoA-I_{Milano} and 1-palmitoyl-2-oleoyl phosphatidylcholine complexes, protected the myocardium in rabbit hearts subjected to global ischemia, followed by reperfusion, and this positive effect was associated with a lowering of lipid hydroperoxide formation, indicating that, in these experimental conditions, ETC-216 worked through an antioxidant mechanism (Marchesi et al., 2008a). Furthermore, Marchesi et al. (2004) published a study showing that ETC-216 was able to significantly reduced the infarct size of rabbits undergoing regional ischemia/reperfusion injury. In addition, electron microscopic analysis revealed that ETC-216 noticeably lessened the alterations of the sarcomere structure and the swelling of mitochondria observed in ischemic vehicle-treated hearts. It is important to highlight that: 1) the ETC-216 minimal effective dose was of 3 mg/kg, which is dramatically lower than those used in preclinical studies of atherosclerosis regression (Ameli et al., 1994; Soma et al., 1995a); and 2) this dose did not modify the plasma lipid/lipoprotein profile, indicating that this cardioprotection occurred independently by a measurable cholesterol mobilization (Marchesi et al., 2004). Later, short-term infusion of ETC-216 acutely reversed aortic valve morphology and histopathology in an experimental model of aortic valve stenosis (Speidl et al., 2010). Besides, valve-leaflet thickening, inflammation, and calcification were dramatically lowered compared with levels measured in the control animals. Furthermore, in vitro studies, performed in cultured porcine aortic valve myofibroblasts exposed to ox-LDL, demonstrated that intracellular cholesterol content, NF- κ B translocation, MCP-1 production, and alkaline phosphatase expression and activity were markedly decreased by ETC-216 treatment (Speidl et al., 2010). Together, these data identify ETC-216 as a potential therapeutic agent in the treatment of aortic valve degeneration and uncover potential mechanistic pathways by which this agent acts on the aortic valve (Speidl et al., 2010).

Translating reports in animal models, a small early human study was performed to evaluate the ability of two different doses (15 and 45 mg/kg) of ETC-216 to reduce coronary plaque burden. In this double-blind trial, five weekly intravenous infusions of ETC-216 resulted in a significant reduction of atheroma volume compared with baseline, and this regression was associated with reverse remodeling of the arterial wall (Nissen et al., 2003; Nicholls et al., 2006; Cinquetti et al., 2008). Greater regression was observed in the 10-mm segments that contained the greatest amount of plaque at baseline (i.e., 10-mm most-diseased segments), suggesting the potential for a greater effect in the areas possibly containing the most amount of lipid. No differences

in efficacy were observed between the two dosing groups. Not surprisingly, these findings were observed in the absence of any increase in steady-state levels of HDL cholesterol (Nissen et al., 2003). Although the ETC-216 compound was well tolerated, some patients were withdrawn from the study for a symptomatic transaminase elevation more than three times the upper limit of normal or the development of diaphoresis and nausea (Bisgaier et al., 2016). Optimization of the manufacturing process led to a newer formulation, called MDCO-216 (Kallend et al., 2016; Parolini et al., 2019). MDCO-216 does not trigger an immune response (Reijers et al., 2017), in contrast to what was previously observed with ETC-216 (Badi et al., 2009; Huang et al., 2009), but it retained the favorable activities on cholesterol efflux and inflammation (Kallend et al., 2016; Kempen et al., 2016). Based on these data, a proof-of-concept, double-blind, placebo-controlled, randomized trial, the MILANO-PILOT study, was designed to evaluate, by intravascular ultrasound (IVUS), the efficacy of MDCO-216 on atherosclerotic plaque burden in patients with an acute coronary syndrome on intensive statin therapy (Marchesi et al., 2011a; Nicholls et al., 2018). After five weekly MDCO-216 infusions (20 mg/kg), the percent atheroma volume, which was adjusted for baseline values, decreased 0.94% with the placebo and 0.21% with MDCO-216. In the 10-mm most-diseased segments, the atheroma plaque volume decreased by 2.2 mm³ in MDCO-216-treated patients and by 1.8 mm³ in the placebo group ($P = 0.83$). Thus, treatment with MDCO-216 was not associated with a significant plaque regression (Nicholls et al., 2018). Therefore, even though the safety profile was excellent, the pharmaceutical company announced discontinuation of the clinical development program for this compound; however, considering the plaque regression observed in the 10-mm most-diseased segment and the low number of patients enrolled in this clinical study, it can be speculated that, with a higher number of patients, the statistical significance could be reached. In addition, the endpoint of coronary atheroma volume evaluated in this study by conventional grayscale IVUS does not provide insight into all aspects of plaque vulnerability and stability that can be modulated by sHDL-apoA-I_M (Speidl et al., 2010; Ibanez et al., 2012). Spectral analysis of the radiofrequency ultrasound backscatter signal and near-infrared spectroscopy have indeed emerged as new strategies for the quantification of various plaque components, that is, fibrotic, fibrofatty, necrotic, and calcific components, that provide incremental risk prediction information, independent of plaque burden (Brown et al., 2015; Honda et al., 2017; Di Bartolo et al., 2018). Lastly, the dose of 20 mg/kg used in this study should be not the “best choice” based on the U-shaped dose-response curve characteristic of the sHDL (Parolini et al., 2019; Pownall and Gotto, 2019) and on the data from the Nissen study where the lowest dose (i.e., 15 mg/kg) was the most efficacious (Fig. 2) (Nissen et al., 2003). Consistent with these speculative hypotheses, a recent study described an alternative method of apoA-I_M production by using genetically modified rice plants. Briefly, seeds from transgenic rice lines were collected, dehusked, whitened, and ground to obtain flour capable of forming a suspension in water. The flour was liquefied at 90°C for 30 minutes in a protease-free saline solution containing α -amylase and then lyophilized, as indicated in patent no. PCT/IB2006/054948. For in vivo experiments,

Fig. 2. Change from baseline in plaque atheroma volume (cubic millimeters) at the 10-mm most-diseased segment evaluated by IVUS in patients treated with placebo or ETC-216 (ETC-216 study) or MDCO-216 (Milano-Pilot Study).

rice-seed protein extract (i.e., rice-milk-apoA-I_M) was resuspended in sterile water. The authors demonstrated that oral administration of rice-milk-apoA-I_M was associated with a significant reduction in the plaque burden in atherosclerosis-susceptible mice (Parolini et al., 2014b; Romano et al., 2018). Moreover, the rice-milk-apoA-I_M displayed both in vitro and in vivo anti-inflammatory properties (Romano et al., 2018). It is important to underline that these results were achieved using: 1) a very low dose of apoA-I_M (i.e., 0.83 mg/kg); and 2) oral gavage administration, an unconventional way of delivery of recombinant proteins. In line with these data, it is worthy to mention two experimental studies demonstrating that MDCO-216 displayed positive effects, even when administered by intraperitoneal injection. Specifically, in an experimental mouse model of nonischemic heart failure (i.e., heart failure induced by transverse aortic constriction), intraperitoneal administration of MDCO-216 reversed pathologic remodeling induced by pressure overload. These beneficial effects observed in MDCO-216-treated mice are proven by: 1) the increase of relative vascularity, 2) the regression of interstitial fibrosis, 3) the improved diastolic function, 4) the decrease of atrial weight, and 5) the normalization of wet lung weight. Consistent with previous data, MDCO-216 exerted these effects without changing HDL cholesterol levels (Aboumsallem et al., 2018). Similar results were obtained when MDCO-216 was infused, by intraperitoneal injection, in mice where heart failure was induced by a specific dietary treatment (Mishra et al., 2018; Aboumsallem et al., 2019).

Other Low-Grade Inflammatory Disease

Cancer. Cancer is the second leading cause of death worldwide and is the result of the stochastic accumulation of spontaneous mutations during DNA replication, combined with environmental exposure and lifestyle habits, both able to significantly influence cancer risk (Tomasetti and Vogelstein, 2015; Global Burden of Disease Cancer Collaboration et al., 2017). The direct correlation between obesity and increased cancer incidence and cancer-related deaths has been widely documented. It has been estimated that 14% of cancer deaths in men and 20% in women are attributable to obesity (Kolb et al., 2016). In the tumor microenvironment, neoplastic and immune cells and microorganisms constitute a dynamic interactive network influenced by genetic makeup and epidemiologic factors, including ageing, diet, smoking,

diabetes mellitus, physical exercise, socioeconomic status, and medication (David et al., 2014; Morgillo et al., 2018; Spranger and Gajewski, 2018). A growing body of evidence suggests that tumor cells require an increased cholesterol supply and can accumulate cholesterol, which is essential for cancer-cell proliferation and tumor progression (Parolini et al., 2013; Mooberry et al., 2016). Consistently, many patients with cancer have reduced plasma levels of cholesterol, which return to normal concentrations on successful remission (Foit et al., 2015). The inCHIANTI clinical trial demonstrated that cancer mortality was significantly increased in community-dwelling older subjects with low HDL cholesterol (<40 mg/dl) concentrations (Zuliani et al., 2017). Moreover, a recent study showed that HDL nanoparticles slowed primary and metastatic tumor growth, together with a reduction of myeloid-derived suppressor cells and Tregs in the metastatic microenvironment that were specifically mediated by SR-BI expression (Rigamonti et al., 2010; Plebanek et al., 2018). These results brought to light that sHDL-targeting SR-BI could represent a new delivery system to carry cancer chemotherapeutic drugs into the tumor cells (McConathy et al., 2008; Shen et al., 2018). In line with this approach, sHDL-apoA-I_M containing a natural topoisomerase I inhibitor, i.e., 10-hydroxycamptothecin, proved efficacy at improving release profile selectivity and cytotoxicity in both in vitro and in vivo experiments (Zhang and Chen, 2010).

Sepsis. Sepsis is a systemic inflammatory syndrome that is caused by an unbalanced host response to an infection (Singer et al., 2016). Nowadays, there is still disagreement between progress in the understanding of the pathophysiology of sepsis and failure of the development of targeted therapies aimed at modifying the disrupted host response (Cohen et al., 2015). In addition, it is important to keep in mind that during sepsis there is increased inflammation and/or immune suppression, as well as a reorganization of the immune and metabolic cell processes (van der Poll et al., 2017). Sepsis is one of the most common causes of death among hospitalized patients in the intensive care unit (Novosad et al., 2016). Obesity has been associated with increased risk of recurrent, nosocomial, and secondary infections, leading to sepsis, renal failure, and mortality. Considerable evidence supports a direct association between acute bacterial or viral infections and increased risk of myocardial infarction (Musher et al., 2019). Our body possesses various endogenous tools to counteract exaggerated responses to infections, including inhibitory lipopolysaccharide-binding proteins and plasma lipoproteins (Tiniakou et al., 2015). During sepsis, however, marked alterations in lipid metabolism are observed. Specifically, HDL and LDL cholesterol levels decrease, whereas very low-density lipoprotein increases and chylomicrons remain unchanged (van Leeuwen et al., 2003; Caligari et al., 2006). Moreover, it has been observed that low-plasma HDL cholesterol concentration (associated with low plasma apoA-I level) is a poor prognostic factor for severe sepsis (Trinder et al., 2019).

The first evidence of protection from endotoxins by HDL came from studies in transgenic mice. Overexpression of human apoA-I-protected mice from a lethal dose of Gram-negative bacterial endotoxin lipopolysaccharide (LPS) increased LPS binding to HDL and decreased TNF- α production (Levine et al., 1993; Parolini et al., 2014a). On the other hand,

using apoA-I null mice as a model for low circulating HDL, it was found that a deficiency in HDL leads to increased susceptibility to cecal ligation and puncture-induced septic death, as well both decreased LPS neutralization and LPS clearance (Guo et al., 2013; Chiesa et al., 2016; Busnelli et al., 2018b). Moreover, administration of reconstituted HDL, containing apoA-I or apoA-I mimetic peptide, to LPS-challenged animals reduced endotoxic animal death, alleviated LPS-induced inflammatory cytokine production, and improved cardiac function (Levine et al., 1993; Quezado et al., 1995; Wait et al., 2005; Parolini et al., 2012b). Coherent with these data, in a rat model of endotoxin-induced endotoxemia, pre-treatment with sHDL-apoA-I_M significantly attenuated liver and renal dysfunction and lung injury and reduced the expression of TNF- α , IL-1 β , IL-6, and adhesion molecule ICAM-1. Moreover, sHDL-apoA-I_M treatment inhibited lipid peroxidation and enhanced total antioxidant capacity (Zhang et al., 2015).

Brain Disease. Increasing evidence suggests that obese patients are more susceptible to the development of mild cognitive impairment, dementia, and Alzheimer disease (AD). Studies have identified several overlapping neurodegenerative mechanisms, including oxidative stress, mitochondrial dysfunction, and inflammation, that are observed in these disorders (Pugazhenthil et al., 2017). A strong correlation between diabetes and AD was first reported in a large population-based Rotterdam study (Ott et al., 1999). The Baltimore Longitudinal Study of Aging reported a higher midlife body mass index associated with an early onset AD and a greater burden of AD pathology (Chuang et al., 2016). Also, consumption of diets high in fat and sugar influences the microbiota composition, which may lead to an imbalanced microbial population in the gut (Busnelli et al., 2018b; Parolini, 2019a). It has been recently hypothesized that the gut microbiota could be part of a mechanistic link between the consumption of high-fat and other unbalanced diets and impaired cognition, termed *the gut-brain axis* (Solas et al., 2017). Various data also suggest that lipids, apoE, and apoA-I influence the processing, accumulation, and clearance of parenchymal β amyloid ($A\beta$) (Loera-Valencia et al., 2019). Specifically, carriers of APOE ϵ 4 allele are at higher risk of developing AD and cerebral amyloid angiopathy (Greenberg et al., 1995), whereas both in vitro and in vivo studies demonstrated that apoA-I has the ability to bind and prevent $A\beta$ aggregation and toxicity (Paula-Lima et al., 2009; Lewis et al., 2010). Based on these data, sHDL containing apoA-I was acutely administered to a mouse model of AD, that is, the amyloid precursor protein presenilin in mice. A reduction in soluble $A\beta$ levels, in the absence of a clear change in the amount of cortical amyloid deposited, was observed (Robert et al., 2016). On the contrary, in vivo chronic administration of recombinant apoA-I_M in a different model of AD significantly reduced the cerebral $A\beta$ concentrations and the cerebral amyloid angiopathy load, together with lower levels of IL-1 β compared with saline-treated mice (Fernández-de Retana et al., 2017). These effects were detected without any change in the lipid profile and using lower doses of recombinant apoA-I_M compared with that tested in previous studies evaluating the antiatherosclerotic efficacy (i.e., 2 mg/kg versus 5 mg/kg) (Kaul et al., 2003; Chiesa et al., 2008; Parolini et al., 2008; Nicholls et al., 2018). We can speculate that apoA-I_M could represent a better therapeutic candidate for brain

protection compared with apoA-I, although further studies are needed to prove the clinical efficacy.

Conclusion

Together, these data strongly support the growing evidence that HDL biology has more to do with the achievement of a steady state rather than with the absolute levels of HDL cholesterol (Karalis and Jukema, 2018). Moreover, based on the newest data, we think apoA-I_M could represent a big challenge toward a new era of a “functional food- and drug-delivery system,” thus offering a new approach toward disease prevention and treatment. With these aims, further investigations are required to strengthen the new data obtained with rice-milk-apoA-I_M, as well as with sHDL-apoA-I_M-containing drugs. The imminent availability of large cohort studies assessing the interplay between diets, gut microbiota, and inflammatory-based disease will give us an appealing opportunity to develop new therapeutic strategies, particularly in the context of a global aging population.

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Participated in research design: Parolini.

Performed data analysis: Parolini.

Wrote or contributed to the writing of the manuscript: Parolini.

References

- Aboumsallem JP, Mishra M, Amin R, Muthuramu I, Kempen H, and De Geest B (2018) Successful treatment of established heart failure in mice with recombinant HDL (Milano). *Br J Pharmacol* **175**:4167–4182.
- Aboumsallem JP, Muthuramu I, Mishra M, Kempen H, and De Geest B (2019) Effective treatment of diabetic cardiomyopathy and heart failure with reconstituted HDL (Milano) in mice. *Int J Mol Sci* **20**:pii: E1273.
- Ameli S, Hultgardh-Nilsson A, Cercek B, Shah PK, Forrester JS, Ageland H, and Nilsson J (1994) Recombinant apolipoprotein A-I Milano reduces intimal thickening after balloon injury in hypercholesterolemic rabbits. *Circulation* **90**:1935–1941.
- Ayala A, Muñoz MF, and Argüelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxid Med Cell Longev* **2014**:360438.
- Bäck M, Yurdagül A Jr, Tabas I, Öörni K, and Kovanen PT (2019) Inflammation and its resolution in atherosclerosis: mediators and therapeutic opportunities. *Nat Rev Cardiol* **16**:389–406.
- Badi I, Cinquetti R, Frascoli M, Parolini C, Chiesa G, Taramelli R, and Acquati F (2009) Intracellular ANKRD1 protein levels are regulated by 26S proteasome-mediated degradation. *FEBS Lett* **583**:2486–2492.
- Badimon JJ, Badimon L, and Fuster V (1990) Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J Clin Invest* **85**:1234–1241.
- Barter PJ, Nicholls S, Rye KA, Anantharamaiah GM, Navab M, and Fogelman AM (2004) Antiinflammatory properties of HDL. *Circ Res* **95**:764–772.
- Bisgaier CL, Ackermann R, Rea T, Rodriguez WV, and Hartman D (2016) ApoA-I-Milano phospholipid complex (ETC-216) infusion in human volunteers. Insights into the phenotypic characteristics of ApoA-I-Milano carriers. *Pharmacol Res* **111**:86–99.
- Brown AJ, Obaid DR, Costopoulos C, Parker RA, Calvert PA, Teng Z, Hoole SP, West NE, Goddard M, and Bennett MR (2015) Direct comparison of virtual-histology intravascular ultrasound and optical coherence tomography imaging for identification of thin-cap fibroatheroma. *Circ Cardiovasc Imaging* **8**:e003487.
- Busnelli M, Manzini S, Hilvo M, Parolini C, Ganzetti GS, Dellera F, Ekroos K, Jänis M, Escalante-Alcalde D, Sirtori CR, et al. (2017) Liver-specific deletion of the Plpp3 gene alters plasma lipid composition and worsens atherosclerosis in apoE^{-/-} mice. *Sci Rep* **7**:44503.
- Busnelli M, Manzini S, Parolini C, Escalante-Alcalde D, and Chiesa G (2018a) Lipid phosphate phosphatase 3 in vascular pathophysiology. *Atherosclerosis* **271**:156–165.
- Busnelli M, Manzini S, Sirtori CR, Chiesa G, and Parolini C (2018b) Effects of vegetable proteins on hypercholesterolemia and gut microbiota modulation. *Nutrients* **10**:E1249.
- Caligari S, Chiesa G, Johnson SK, Camisassi D, Gilio D, Marchesi M, Parolini C, Rubio LA, and Sirtori CR (2006) Lupin (Lupinus albus) protein isolate (L-ISO) has adequate nutritional value and reduces large intestinal weight in rats after restricted and ad libitum feeding. *Ann Nutr Metab* **50**:528–537.

- Chiesa G, Busnelli M, Manzini S, and Parolini C (2016) Nutraceuticals and bioactive components from fish for dyslipidemia and cardiovascular risk reduction. *Mar Drugs* **14**:E113.
- Chiesa G, Parolini C, Canavesi M, Colombo N, Sirtori CR, Fumagalli R, Franceschini G, and Bernini F (1998) Human apolipoproteins A-I and A-II in cell cholesterol efflux: studies with transgenic mice. *Arterioscler Thromb Vasc Biol* **18**: 1417–1423.
- Chiesa G, Parolini C, and Sirtori CR (2008) Acute effects of high-density lipoproteins: biochemical basis and clinical findings. *Curr Opin Cardiol* **23**:379–385.
- Chiesa G, Rigamonti E, Monteggia E, Parolini C, Marchesi M, Miragoli L, Grotti A, Maggioni F, Lorusso V, and Sirtori CR (2004) Evaluation of a soft atherosclerotic lesion in the rabbit aorta by an invasive IVUS method versus a non-invasive MRI technology. *Atherosclerosis* **174**:25–33.
- Chuang YF, An Y, Bilgel M, Wong DF, Troncoso JC, O'Brien RJ, Breitner JC, Ferrucci L, Resnick SM, and Thambisetty M (2016) Midlife adiposity predicts earlier onset of Alzheimer's dementia, neuropathology and presymptomatic cerebral amyloid accumulation. *Mol Psychiatry* **21**:910–915.
- Cinqueti R, Badi I, Campione M, Bortoletto E, Chiesa G, Parolini C, Camesasca C, Russo A, Taramelli R, and Acquatì F (2008) Transcriptional deregulation and a missense mutation define ANKRD1 as a candidate gene for total anomalous pulmonary venous return. *Hum Mutat* **29**:468–474.
- Cohen J, Vincent JL, Adhikari NK, Machado FR, Angus DC, Calandra T, Jaton K, Giulieri S, Delaloye J, Opal S, et al. (2015) Sepsis: a roadmap for future research. *Lancet Infect Dis* **15**:581–614.
- David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, et al. (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**:559–563.
- Dellera F, Ganzetti GS, Froio A, Manzini S, Busnelli M, Meintzer A, Sirtori CR, Chiesa G, and Parolini C (2016) L-homoarginine administration reduces neointimal hyperplasia in balloon-injured rat carotids. *Thromb Haemostasis* **116**:400–402.
- Di Bartolo BA, Psaltis PJ, Bursill CA, and Nicholls SJ (2018) Translating evidence of HDL and plaque regression. *Arterioscler Thromb Vasc Biol* **38**: 1961–1968.
- Ertunc ME and Hotamisligil GS (2016) Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. *J Lipid Res* **57**:2099–2114.
- Feingold KR and Grunfeld C (2011) The role of HDL in innate immunity. *J Lipid Res* **52**:1–3.
- Fernández-de Retana S, Montañola A, Marazuela P, De La Cuesta M, Batlle A, Fatar M, Grudzenski S, Montaner J, and Hernández-Guillamon M (2017) Intravenous treatment with human recombinant ApoA-I Milano reduces beta amyloid cerebral deposition in the APP23-transgenic mouse model of Alzheimer's disease. *Neurobiol Aging* **60**:116–128.
- Florentin M, Liberopoulos EN, Wierzbicki AS, and Mikhailidis DP (2008) Multiple actions of high-density lipoprotein. *Curr Opin Cardiol* **23**:370–378.
- Foit L, Giles FJ, Gordon LI, and Thaxton CS (2015) Synthetic high-density lipoprotein-like nanoparticles for cancer therapy. *Expert Rev Anticancer Ther* **15**: 27–34.
- Franceschini G, Calabresi L, Chiesa G, Parolini C, Sirtori CR, Canavesi M, and Bernini F (1999) Increased cholesterol efflux potential of sera from ApoA-I-Milano carriers and transgenic mice. *Arterioscler Thromb Vasc Biol* **19**:1257–1262.
- Frydrych LM, Bian G, O'Lone DE, Ward PA, and Delano MJ (2018) Obesity and type 2 diabetes mellitus drive immune dysfunction, infection development, and sepsis mortality. *J Leukoc Biol* **104**:525–534.
- Ganzetti GS, Rigamonti E, Brambilla R, and Parolini C (2019) Microarray analysis of liver gene expression in apoA-I and apoA-IMilano knock-in mouse models. *Clin Res Nephrol Kidney Dis* **1**:1001.
- Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, Dicker DJ, Chimed-Orchir O, Dandona R, Dandona L, et al.; Global Burden of Disease Cancer Collaboration (2017) Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the Global Burden of Disease Study. *JAMA Oncol* **3**:524–548.
- Gordon SM, Hofmann S, Askew DS, and Davidson WS (2011) High density lipoprotein: it's not just about lipid transport anymore. *Trends Endocrinol Metab* **22**:9–15.
- Greenberg SM, Rebeck GW, Vonsattel JP, Gomez-Isla T, and Hyman BT (1995) Apolipoprotein E epsilon 4 and cerebral hemorrhage associated with amyloid angiopathy. *Ann Neurol* **38**:254–259.
- Grunfeld C and Feingold KR (2008) HDL and innate immunity: a tale of two apolipoproteins. *J Lipid Res* **49**:1605–1606.
- Guo L, Ai J, Zheng Z, Howatt DA, Daugherty A, Huang B, and Li XA (2013) High density lipoprotein protects against polymicrobe-induced sepsis in mice. *J Biol Chem* **288**:17947–17953.
- Herrington W, Lacey B, Sherlik P, Armitage J, and Lewington S (2016) Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. *Circ Res* **118**:535–546.
- Honda S, Sidharta SL, Shishikura D, Takata K, Di Giovanni GA, Nguyen T, Janssan A, Kim SW, Andrews J, Psaltis PJ, et al. (2017) High-density lipoprotein cholesterol associated with change in coronary plaque lipid burden assessed by near infrared spectroscopy. *Atherosclerosis* **265**:110–116.
- Hotamisligil GS (2006) Inflammation and metabolic disorders. *Nature* **444**:860–867.
- Huang LY, Dumontelle JL, Zolod M, Deora A, Mozier NM, and Golding B (2009) Use of toll-like receptor assays to detect and identify microbial contaminants in biological products. *J Clin Microbiol* **47**:3427–3434.
- Ibanez B, Giannarelli C, Cimmino G, Santos-Gallego CG, Alique M, Pinero A, Vilahur G, Fuster V, Badimon L, and Badimon JJ (2012) Recombinant HDL(Milano) exerts greater anti-inflammatory and plaque stabilizing properties than HDL(wild-type). *Atherosclerosis* **220**:72–77.
- Ibanez B, Vilahur G, Cimmino G, Speidl WS, Pinero A, Choi BG, Zafar MU, Santos-Gallego CG, Krause B, Badimon L, et al. (2008) Rapid change in plaque size, composition, and molecular footprint after recombinant apolipoprotein A-I Milano (ETC-216) administration: magnetic resonance imaging study in an experimental model of atherosclerosis. *J Am Coll Cardiol* **51**:1104–1109.
- Kallend DG, Reijers JA, Bellibas SE, Bobillier A, Kempen H, Burggraaf J, Moerland M, and Wijngaard PL (2016) A single infusion of MDCO-216 (ApoA-1 Milano/POPC) increases ABCA1-mediated cholesterol efflux and pre-beta 1 HDL in healthy volunteers and patients with stable coronary artery disease. *Eur Heart J Cardiovasc Pharmacother* **2**:23–29.
- Kannel WB, Dawber TR, Friedman GD, Glennon WE, and McNamara PM (1964) Risk factors in coronary heart disease. An evaluation of several serum lipids as predictors of coronary heart disease; the Framingham study. *Ann Intern Med* **61**: 888–899.
- Karalis I and Jukema JW (2018) HDL mimetics infusion and regression of atherosclerosis: is it still considered a valid therapeutic option? *Curr Cardiol Rep* **20**:66.
- Kaul S, Rukshin V, Santos R, Azarbal B, Bisgaier CL, Johansson J, Tsang VT, Chyu KY, Cercek B, Mirocha J, et al. (2003) Intramural delivery of recombinant apolipoprotein A-IMilano/phospholipid complex (ETC-216) inhibits in-stent stenosis in porcine coronary arteries. *Circulation* **107**:2551–2554.
- Kazi DS, Penko JM, and Bibbins-Domingo K (2017) Statins for primary prevention of cardiovascular disease: review of evidence and recommendations for clinical practice. *Med Clin North Am* **101**:689–699.
- Kempen HJ, Asztalos BF, Moerland M, Jeyarajah E, Otvos J, Kallend DG, Bellibas SE, and Wijngaard PL (2016) High-density lipoprotein subfractions and cholesterol efflux capacities after infusion of MDCO-216 (apolipoprotein A-IMilano/palmitoyl-oleoyl-phosphatidylcholine) in healthy volunteers and stable coronary artery disease patients. *Arterioscler Thromb Vasc Biol* **36**:736–742.
- Kolb R, Sutterwala FS, and Zhang W (2016) Obesity and cancer: inflammation bridges the two. *Curr Opin Pharmacol* **29**:77–89.
- Kühnast S, Fiocco M, van der Hoorn JW, Princen HM, and Jukema JW (2015) Innovative pharmaceutical interventions in cardiovascular disease: focusing on the contribution of non-HDL-C/LDL-C-lowering versus HDL-C-raising: a systematic review and meta-analysis of relevant preclinical studies and clinical trials. *Eur J Pharmacol* **763** (Pt A):48–63.
- Levine DM, Parker TS, Donnelly TM, Walsh A, and Rubin AL (1993) In vivo protection against endotoxin by plasma high density lipoprotein. *Proc Natl Acad Sci USA* **90**:12040–12044.
- Lewis TL, Cao D, Lu H, Mans RA, Su YR, Jungbauer L, Linton MF, Fazio S, LaDu MJ, and Li L (2010) Overexpression of human apolipoprotein A-I preserves cognitive function and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer disease. *J Biol Chem* **285**:36958–36968.
- Lindahl M, Petrova J, Dalla-Riva J, Wasserstrom S, Rippe C, Domingo-Espin J, Kotowska D, Krupinska E, Berggreen C, Jones HA, et al. (2015) ApoA-I Milano stimulates lipolysis in adipose cells independently of cAMP/PKA activation. *J Lipid Res* **56**:2248–2259.
- Liu AC, Lawn RM, Verstuyft JG, and Rubin EM (1994) Human apolipoprotein A-I prevents atherosclerosis associated with apolipoprotein[a] in transgenic mice. *J Lipid Res* **35**:2263–2267.
- Loera-Valencia R, Goikolea J, Parrado-Fernandez C, Merino-Serrais P, and Maioli S (2019) Alterations in cholesterol metabolism as a risk factor for developing Alzheimer's disease: potential novel targets for treatment. *J Steroid Biochem Mol Biol* **190**:104–114.
- Mackey RH, Greenland P, Goff DC Jr, Lloyd-Jones D, Sibley CT, and Mora S (2012) High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). *J Am Coll Cardiol* **60**:508–516.
- Madsen CM, Varbo A, and Nordestgaard BG (2017) Extreme high high-density lipoprotein cholesterol is paradoxically associated with high mortality in men and women: two prospective cohort studies. *Eur Heart J* **38**:2478–2486.
- Madsen CM, Varbo A, Tybjaerg-Hansen A, Frikke-Schmidt R, and Nordestgaard BG (2018) U-shaped relationship of HDL and risk of infectious disease: two prospective population-based cohort studies. *Eur Heart J* **39**:1181–1190.
- Manzini S, Busnelli M, Parolini C, Minoli L, Ossoli A, Brambilla E, Simonelli S, Lekka E, Persidis A, Scanziani E, et al. (2019) Topiramate protects apoE-deficient mice from kidney damage without affecting plasma lipids. *Pharmacol Res* **141**: 189–200.
- Manzini S, Pinna C, Busnelli M, Cinquanta P, Rigamonti E, Ganzetti GS, Dellera F, Sala A, Calabresi L, Franceschini G, et al. (2015) Beta2-adrenergic activity modulates vascular tone regulation in leithin:cholesterol acyltransferase knockout mice. *Vasc Res Pharmacol* **74**:114–121.
- Marchesi M, Booth EA, Davis T, Bisgaier CL, and Lucchesi BR (2004) Apolipoprotein A-IMilano and 1-palmitoyl-2-oleoyl phosphatidylcholine complex (ETC-216) protects the in vivo rabbit heart from regional ischemia-reperfusion injury. *J Pharmacol Exp Ther* **311**:1023–1031.
- Marchesi M, Booth EA, Rossoni G, Garcia RA, Hill KR, Sirtori CR, Bisgaier CL, and Lucchesi BR (2008a) Apolipoprotein A-IMilano/POPC complex attenuates post-ischemic ventricular dysfunction in the isolated rabbit heart. *Atherosclerosis* **197**:572–578.
- Marchesi M, Parolini C, Caligari S, Gilio D, Manzini S, Busnelli M, Cinquanta P, Camera M, Brambilla M, Sirtori CR, et al. (2011a) Rosuvastatin does not affect human apolipoprotein A-I expression in genetically modified mice: a clue to the disputed effect of statins on HDL. *Br J Pharmacol* **164**:1460–1468.
- Marchesi M, Parolini C, Diani E, Rigamonti E, Cornelli L, Arnoldi A, Sirtori CR, and Chiesa G (2008b) Hypolipidaemic and anti-atherosclerotic effects of lupin proteins in a rabbit model. *Br J Nutr* **100**:707–710.
- Marchesi M, Parolini C, Valetti C, Mangione P, Obici L, Giorgetti S, Raimondi S, Donadei S, Gregorini G, Merlini G, et al. (2011b) The intracellular quality control system down-regulates the secretion of amyloidogenic apolipoprotein A-I variants: a possible impact on the natural history of the disease. *Biochim Biophys Acta* **1812**: 87–93.

- McConathy WJ, Nair MP, Paranjape S, Mooberry L, and Lacko AG (2008) Evaluation of synthetic/reconstituted high-density lipoproteins as delivery vehicles for paclitaxel. *Anticancer Drugs* **19**:183–188.
- Mishra M, Muthuramu I, Aboumsallem JP, Kempen H, and De Geest B (2018) Reconstituted HDL (Milano) treatment efficaciously reverses heart failure with preserved ejection fraction in mice. *Int J Mol Sci* **19**:pii: E3399.
- Montecucco F, Brauersreuther V, Burger F, Lenglet S, Pelli G, Carbone F, Fraga-Silva R, Stergiopoulos N, Monaco C, Mueller C, et al. (2015) Anti-apoA-1 auto-antibodies increase mouse atherosclerotic plaque vulnerability, myocardial necrosis and mortality triggering TLR2 and TLR4. *Thromb Haemost* **114**:410–422.
- Montecucco F, Vuilleumier N, Pagano S, Lenglet S, Bertolotto M, Brauersreuther V, Pelli G, Kovari E, Pane B, Spinella G, et al. (2011) Anti-Apolipoprotein A-1 auto-antibodies are active mediators of atherosclerotic plaque vulnerability. *Eur Heart J* **32**:412–421.
- Mooberry LK, Sabnis NA, Panchoo M, Nagarajan B, and Lacko AG (2016) Targeting the SR-B1 receptor as a gateway for cancer therapy and imaging. *Front Pharmacol* **7**:466.
- Morgillo F, Dallio M, Della Corte CM, Gravina AG, Viscardi G, Loguercio C, Ciardiello F, and Federico A (2018) Carcinogenesis as a result of multiple inflammatory and oxidative hits: a comprehensive review from tumor microenvironment to gut microbiota. *Neoplasia* **20**:721–733.
- Musher DM, Abers MS, and Corrales-Medina VF (2019) Acute infection and myocardial infarction. *N Engl J Med* **380**:171–176.
- Nicholls SJ, Puri R, Ballantyne CM, Jukema JW, Kastelein JJP, Koenig W, Wright RS, Kallend D, Wijngaard P, Borgman M, et al. (2018) Effect of infusion of high-density lipoprotein mimetic containing recombinant apolipoprotein A-I Milano on coronary disease in patients with an acute coronary syndrome in the MILANO-PILOT trial: a randomized clinical trial. *JAMA Cardiol* **3**:806–814.
- Nicholls SJ, Tuzcu EM, Sipahi I, Schoenhagen P, Crowe T, Kapadia S, and Nissen SE (2006) Relationship between atheroma regression and change in lumen size after infusion of apolipoprotein A-I Milano. *J Am Coll Cardiol* **47**:992–997.
- Nissen SE, Tsunoda T, Tuzcu EM, Schoenhagen P, Cooper CJ, Yasin M, Eaton GM, Lauer MA, Sheldon WS, Grines CL, et al. (2003) Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA* **290**:2292–2300.
- Novosad SA, Sapiano MR, Grigg C, Lake J, Robyn M, Dumyati G, Felsen C, Blog D, Dufort E, Zansky S, et al. (2016) Vital signs: epidemiology of sepsis: prevalence of health care factors and opportunities for prevention. *MMWR Morb Mortal Wkly Rep* **65**:864–869.
- Ott A, Stolk RP, van Harskamp F, Pols HA, Hofman A, and Breteler MM (1999) Diabetes mellitus and the risk of dementia: the Rotterdam Study. *Neurology* **53**:1937–1942.
- Parolini C (2019a) Effects of fibers and gut microbiota on low-grade inflammatory human disease. *Hepatobiliary Surg Nutr* (Epub ahead of print.).
- Parolini C (2019b) Effects of fish n-3 PUFAs on intestinal microbiota and immune system. *Mar Drugs* **17**:E374.
- Parolini C, Adorni MP, Busnelli M, Manzini S, Cipollari E, Favari E, Lorenzon P, Ganzetti GS, Fingerle J, Bernini F, et al. (2019) Infusions of large synthetic HDL containing trimeric apoA-I stabilize atherosclerotic plaques in hypercholesterolemic rabbits. *Can J Cardiol* **35**:1400–1408.
- Parolini C, Bjørndal B, Busnelli M, Manzini S, Ganzetti GS, Dellera F, Ramsvik M, Bruheim I, Berge RK, and Chiesa G (2017) Effect of dietary components from antarctic krill on atherosclerosis in apoE-deficient mice. *Mol Nutr Food Res* **61**:12.
- Parolini C, Busnelli M, Ganzetti GS, Dellera F, Manzini S, Scanziani E, Johnson JL, Sirtori CR, and Chiesa G (2014a) Magnetic resonance imaging visualization of vulnerable atherosclerotic plaques at the brachiocephalic artery of apolipoprotein E knockout mice by the blood-pool contrast agent B22956/1. *Mol Imaging* **13**:1–9.
- Parolini C, Caligari S, Gilio D, Manzini S, Busnelli M, Montagnani M, Locatelli M, Diani E, Giavarini F, Caruso D, et al. (2012a) Reduced biliary sterol output with no change in total faecal excretion in mice expressing a human apolipoprotein A-I variant. *Liver Int* **32**:1363–1371.
- Parolini C, Chiesa G, Gong E, Caligari S, Cortese MM, Koga T, Forte TM, and Rubin EM (2005) Apolipoprotein A-I and the molecular variant apoA-I(Milano): evaluation of the antiatherogenic effects in knock-in mouse model. *Atherosclerosis* **183**:222–229.
- Parolini C, Chiesa G, Zhu Y, Forte T, Caligari S, Gianazza E, Sacco MG, Sirtori CR, and Rubin EM (2003) Targeted replacement of mouse apolipoprotein A-I with human ApoA-I or the mutant ApoA-IMilano. Evidence of ApoA-IM impaired hepatic secretion. *J Biol Chem* **278**:4740–4746.
- Parolini C, Manzini S, Busnelli M, Rigamonti E, Marchesi M, Diani E, Sirtori CR, and Chiesa G (2013) Effect of the combinations between pea proteins and soluble fibres on cholesterolaemia and cholesterol metabolism in rats. *Br J Nutr* **110**:1394–1401.
- Parolini C, Marchesi M, and Chiesa G (2009) HDL therapy for the treatment of cardiovascular diseases. *Curr Vasc Pharmacol* **7**:550–556.
- Parolini C, Marchesi M, Lorenzon P, Castano M, Balconi E, Miragoli L, Chaabane L, Morisetti A, Lorusso V, Martin BJ, et al. (2008) Dose-related effects of repeated ETC-216 (recombinant apolipoprotein A-I Milano/1-palmitoyl-2-oleoyl phosphatidylcholine complexes) administrations on rabbit lipid-rich soft plaques: in vivo assessment by intravascular ultrasound and magnetic resonance imaging. *J Am Coll Cardiol* **51**:1098–1103.
- Parolini C, Rigamonti E, Marchesi M, Busnelli M, Cinquanta P, Manzini S, Sirtori CR, and Chiesa G (2012b) Cholesterol-lowering effect of dietary Lupinus angustifolius proteins in adult rats through regulation of genes involved in cholesterol homeostasis. *Food Chem* **132**:1475–1479.
- Parolini C, Vik R, Busnelli M, Bjørndal B, Holm S, Brattelid T, Manzini S, Ganzetti GS, Dellera F, Halvorsen B, et al. (2014b) A salmon protein hydrolysate exerts lipid-independent anti-atherosclerotic activity in ApoE-deficient mice. *PLoS One* **9**:e97598.
- Paula-Lima AC, Tricerri MA, Brito-Moreira J, Bomfim TR, Oliveira FF, Magdesian MH, Grinberg LT, Panizzutti R, and Ferreira ST (2009) Human apolipoprotein A-I binds amyloid-beta and prevents Abeta-induced neurotoxicity. *Int J Biochem Cell Biol* **41**:1361–1370.
- Penson P, Long DL, Howard G, Howard VJ, Jones SR, Martin SS, Mikhailidis DP, Muntner P, Rizzo M, Rader DJ, et al. (2019) Associations between cardiovascular disease, cancer, and very low high-density lipoprotein cholesterol in the REasons for Geographical and Racial Differences in Stroke (REGARDS) study. *Cardiovasc Res* **115**:204–212.
- Plebanek MP, Bhaumik D, and Thaxton CS (2018) HDL and the golden key to cancer immunity? *Oncoscience* **5**:164–166.
- Pownall HJ and Gotto AM Jr (2019) Cholesterol: can't live with it, can't live without it. *Methodist DeBakey Cardiovasc J* **15**:9–15.
- Pugazhenth S, Qin L, and Reddy PH (2017) Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. *Biochim Biophys Acta Mol Basis Dis* **1863**:1037–1045.
- Quezado ZM, Natanson C, Banks SM, Alling DW, Koev CA, Danner RL, Elin RJ, Hosseini JM, Parker TS, Levine DM, et al. (1995) Therapeutic trial of reconstituted human high-density lipoprotein in a canine model of gram-negative septic shock. *J Pharmacol Exp Ther* **272**:604–611.
- Reijers JAA, Kallend DG, Malone KE, Jukema JW, Wijngaard PLJ, Burggraaf J, and Moerland M (2017) MDCO-216 does not induce adverse immunostimulation, in contrast to its predecessor ETC-216. *Cardiovasc Drugs Ther* **31**:381–389.
- Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriaga E, et al.; CIRT Investigators (2019) Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med* **380**:752–762.
- Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, et al.; CANTOS Trial Group (2017) Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* **377**:1119–1131.
- Rigamonti E, Parolini C, Marchesi M, Diani E, Brambilla S, Sirtori CR, and Chiesa G (2010) Hypolipidemic effect of dietary pea proteins: impact on genes regulating hepatic lipid metabolism. *Mol Nutr Food Res* **54** (Suppl 1):S24–S30.
- Robert J, Stukas S, Button E, Cheng WH, Lee M, Fan J, Wilkinson A, Kulic I, Wright SD, and Wellington CL (2016) Reconstituted high-density lipoproteins acutely reduce soluble brain A β levels in symptomatic APP/PS1 mice. *Biochim Biophys Acta* **1862**:1027–1036.
- Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, et al. (2014) HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med* **371**:2383–2393.
- Romano G, Reggi S, Kutryb-Zajac B, Facchetti A, Chisci E, Pettinato M, Giuffrè MR, Vecchio F, Leoni S, De Giorgi M, et al. (2018) APOA-1Milano muteins, orally delivered via genetically modified rice, show anti-atherogenic and anti-inflammatory properties in vitro and in ApoE^{-/-} atherosclerotic mice. *Int J Cardiol* **271**:233–239.
- Shah PK, Nilsson J, Kaul S, Fishbein MC, Ageland H, Hamsten A, Johansson J, Karpe F, and Cercek B (1998) Effects of recombinant apolipoprotein A-I(Milano) on aortic atherosclerosis in apolipoprotein E-deficient mice. *Circulation* **97**:780–785.
- Shen WJ, Azhar S, and Kraemer FB (2018) SR-B1: a unique multifunctional receptor for cholesterol influx and efflux. *Annu Rev Physiol* **80**:95–116.
- Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche JD, Coopersmith CM, et al. (2016) The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* **315**:801–810.
- Solas M, Milagro FI, Ramírez MJ, and Martínez JA (2017) Inflammation and gut-brain axis link obesity to cognitive dysfunction: plausible pharmacological interventions. *Curr Opin Pharmacol* **37**:87–92.
- Soma MR, Donetti E, Parolini C, Barberi L, Paoletti R, Fumagalli R, and Catapano AL (1994) Effect of lacidipine on the carotid intimal hyperplasia induced by cuff injury. *J Cardiovasc Pharmacol* **23** (Suppl 5):S71–S74.
- Soma MR, Donetti E, Parolini C, Mazzini G, Ferrari C, Fumagalli R, and Paoletti R (1993) HMG CoA reductase inhibitors: in vivo effects on carotid intimal thickening in normocholesterolemic rabbits. *Arterioscler Thromb* **13**:571–578.
- Soma MR, Donetti E, Parolini C, Sirtori CR, Fumagalli R, and Franceschini G (1995a) Recombinant apolipoprotein A-IMilano dimer inhibits carotid intimal thickening induced by perivascular manipulation in rabbits. *Circ Res* **76**:405–411.
- Soma MR, Parolini C, Donetti E, Fumagalli R, and Paoletti R (1995b) Inhibition of isoprenoid biosynthesis and arterial smooth-muscle cell proliferation. *J Cardiovasc Pharmacol* **25** (Suppl 4):S20–S24.
- Sorci-Thomas MG and Thomas MJ (2012) High density lipoprotein biogenesis, cholesterol efflux, and immune cell function. *Arterioscler Thromb Vasc Biol* **32**:2561–2565.
- Speidl WS, Cimmino G, Ibanez B, Elmariah S, Hutter R, Garcia MJ, Fuster V, Goldman ME, and Badimon JJ (2010) Recombinant apolipoprotein A-I Milano rapidly reverses aortic valve stenosis and decreases leaflet inflammation in an experimental rabbit model. *Eur Heart J* **31**:2049–2057.
- Spranger S and Gajewski TF (2018) Impact of oncogenic pathways on evasion of antitumour immune responses. *Nat Rev Cancer* **18**:139–147.
- Talbot CPJ, Plat J, Ritsch A, and Mensink RP (2018) Determinants of cholesterol efflux capacity in humans. *Prog Lipid Res* **69**:21–32.
- Tiniakou I, Drakos E, Sinatkas V, Van Eck M, Zannis VI, Boumpas D, Verginis P, and Kardassis D (2015) High-density lipoprotein attenuates Th1 and Th17 auto-immune responses by modulating dendritic cell maturation and function. *J Immunol* **194**:4676–4687.
- Tomasetti C and Vogelstein B (2015) Cancer etiology: variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science* **347**:78–81.
- Trinder M, Boyd JH, and Brunham LR (2019) Molecular regulation of plasma lipid levels during systemic inflammation and sepsis. *Curr Opin Lipidol* **30**:108–116.

- van der Poll T, van de Veerdonk FL, Scicluna BP, and Netea MG (2017) The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol* **17**:407–420.
- van Leeuwen HJ, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, and van Kessel KP (2003) Lipoprotein metabolism in patients with severe sepsis. *Crit Care Med* **31**:1359–1366.
- Vik R, Busnelli M, Parolini C, Bjørndal B, Holm S, Bohov P, Halvorsen B, Brattelid T, Manzini S, Ganzetti GS, et al. (2013) An immunomodulating fatty acid analogue targeting mitochondria exerts anti-atherosclerotic effect beyond plasma cholesterol-lowering activity in apoe(-/-) mice. *PLoS One* **8**:e81963.
- Voight BF, Peloso GM, Orho-Melander M, Frikke-Schmidt R, Barbalic M, Jensen MK, Hindy G, Hólm H, Ding EL, Johnson T, et al. (2012) Plasma HDL cholesterol and risk of myocardial infarction: a mendelian randomisation study. *Lancet* **380**: 572–580.
- Wait R, Chiesa G, Parolini C, Miller I, Begum S, Brambilla D, Galluccio L, Ballerio R, Eberini I, and Gianazza E (2005) Reference maps of mouse serum acute-phase proteins: changes with LPS-induced inflammation and apolipoprotein A-I and A-II transgenes. *Proteomics* **5**:4245–4253.
- Zhang X and Chen B (2010) Recombinant high density lipoprotein reconstituted with apolipoprotein AI cysteine mutants as delivery vehicles for 10-hydroxycamptothecin. *Cancer Lett* **298**:26–33.
- Zhang X, Wang L, and Chen B (2015) Recombinant HDL (Milano) protects endotoxin-challenged rats from multiple organ injury and dysfunction. *Biol Chem* **396**:53–60.
- Zuliani G, Volpato S, Dugo M, Vigna GB, Morieri ML, Maggio M, Cherubini A, Bandinelli S, Guralnik JM, and Ferrucci L (2017) Combining LDL-C and HDL-C to predict survival in late life: the InChianti study. *PLoS One* **12**:e0185307.
- PCT/IB2006/054948. 2008, Fogher C., Reggi S., and Perfanov K. IN-PLANT PRODUCTION OF DIMERIC AND/OR OLIGOMERIC (COMPRISING THREE OR MORE UNITS) FORMS OF HUMAN APO A-1 PROTEIN MUTEINS.

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