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-IMilano (sHDL-apoA-IM), 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-IM 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-IM demonstrated anti-inflammatory and antiatherogenic properties in a mouse model, even though administrated by an unconventional way: by oral gavage. Additionally, recent data have uncovered new therapeutic applications for this sHDL-apoA-IM. This review provides an overview of all potential application of sHDL-apoA-IM in some inflammatory-based diseases.

SIGNIFICANCE STATEMENT

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

ABBREVIATIONS: AD, Alzheimer disease; apoA-IM, apolipoprotein A-I Milano; CVD, cardiovascular disease; ETC-216, sHDL constituted by recombinant apoA-IM 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-kB, nuclear factor-kB; sHDL-apoA-IM, synthetic HDL containing recombinant form of apoA-IM; sPLA2, secretory phospholipase A2; β2 TLR, toll-like-receptor; TNF-α, tumor necrosis factor-α; Tregs, T regulatory cell.
increased risk of premature cardiovascular disease (CVD) 
to proteins (LDL) by inhibiting lipid peroxidation and by removing oxidation products, mainly lipid hydroperoxides, from the LDL (Franceschini et al., 1999; Chiesa et al., 2008; Franceschini et al., 1999; Parolini et al., 2012a), and, recently, 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-β2) 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-kB translocation and can increase expression of heme-oxgenase 1 (Grunfeld and Feingold, 2008). Besides, HDL possesses antioxidant properties because it can 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) is the first variant discovered in 1974 in individuals from Limone sul Garda in northern Italy (Parolini et al., 2003, 2005; Marchesi et al., 2011b). This variant 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 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<sub>M</sub> 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<sub>M</sub> 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<sub>M</sub> 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<sub>M</sub>, promoting the idea that synthetic HDL (sHDL) containing the recombinant form of apoA-I<sub>M</sub> (sHDL-apoA-I<sub>M</sub>) 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-1β (IL-1β) 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-1β, 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<sub>M</sub> (Parolini et al., 2009; Kühnast et al., 2015). Briefly, intravenous infusions of sHDL-apoA-I<sub>M</sub> 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<sub>M</sub> 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<sub>M</sub> administration (Parolini et al., 2008; Ibanez et al., 2012). Moreover, several preclinical data indicated that sHDL-apoA-I<sub>M</sub> is effective in restenosis prevention (Soma et al., 1995a; Dellera et al., 2016). In the earliest study, rabbits fed a high-cholesterol diet were injected with sHDL-apoA-I<sub>M</sub> 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<sub>M</sub>-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-IM was investigated in a porcine model of coronary stenting. Pigs were injected with sHDL-apoA-IM 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-IM-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-IM 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 reduce 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 cardio-protection 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-kB 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 (Bisgaard 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-IM (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; Ponnall 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 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,
rice-seed protein extract (i.e., rice-milk-apoA-IM) was resuspended in sterile water. The authors demonstrated that oral administration of rice-milk-apoA-IM 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-IM 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-IM (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; Moobery 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-IM 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 lipopolysaccaride-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 mimic 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, pretreatment with sHDL-apoA-I\textsubscript{M} significantly attenuated liver and renal dysfunction and lung injury and reduced the expression of TNF-\textalpha, IL-1\beta, IL-6, and adhesion molecule ICAM-1. Moreover, sHDL-apoA-I\textsubscript{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 (Pugazhenthi 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 \beta amyloid (A\beta) (Loera-Valencia et al., 2019). Specifically, carriers of APOE4 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\textsubscript{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\beta 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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{M}, as well as with sHDL-apoA-I\textsubscript{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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**Author Contributions**

**Participants in research design:** Parolini.

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

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