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Hypoxia worsens the impact of intracellular triglyceride accumulation promoted by electronegative low-density lipoprotein in cardiomyocytes by impairing perilipin 5 upregulation

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ABSTRACT

Plasma lipoproteins are a source of lipids for the heart, and the proportion of electronegative low density lipoprotein [LDL(−)] is elevated in cardiometabolic diseases. Perilipin 5 (Plin5) is a crucial protein for lipid droplet management in the heart. Our aim was to assess the effect of LDL(−) on intracellular lipid content and Plin5 levels in cardiomyocytes and to determine whether these effects were influenced by hypoxia. HL-1 cardiomyocytes were exposed to native LDL [LDL(+)], LDL(−), and LDL(+) enriched in non-esterified fatty acids (NEFA) by phospholipase A_2 (PLA_2)-mediated lipolysis [PLA_2-LDL(+)] or by NEFA loading [NEFA-LDL(+)] under normoxia or hypoxia. LDL(−), PLA_2-LDL(+) and NEFA-LDL(+) raised the intracellular NEFA and triglyceride (TG) content of normoxic cardiomyocytes. Plin5 was moderately upregulated by LDL(+) but more highly upregulated by LDL(−), PLA_2-LDL(+) and NEFA-LDL(+) in normoxic cardiomyocytes. Hypoxia enhanced the effect of LDL(−), PLA_2-LDL(+) and NEFA-LDL(+) on intracellular TG and NEFA concentrations but, in contrast, counteracted the upregulatory effect of these LDLs on Plin5. Fluorescence microscopy experiments showed that hypoxic cardiomyocytes exposed to LDL(−), PLA_2-LDL(+) and NEFA-LDL(+) have an increased production of reactive oxygen species (ROS). By treating hypoxic cardiomyocytes with WY-14643 (PPARα agonist), Plin5 remained high. In this situation, LDL(−) failed to enhance intracellular NEFA concentration and ROS production. In conclusion, these results show that Plin5 deficiency in hypoxic cardiomyocytes exposed to LDL(−) dramatically increases the levels of unpacked NEFA and ROS.

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Abbreviations: CE, cholesteryl ester; DHE, dihydroethidium; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine; DM2, type 2 diabetes mellitus; FA, fatty acids; FC, free cholesterol; GGE, non-denaturing gradient gel electrophoresis; LD, lipid droplet; LDL, low-density lipoprotein; LDL(−), electronegative LDL; LDL(+), native LDL; Lp-PLA2, lipoprotein-associated phospholipase A2; NEFA, nonesterified fatty acids; PC, phosphatidylcholine; PLC, phospholipase C; Plin5, perilipin 5; ROS, reactive oxygen species.

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1. Introduction

Myocardial triglyceride (TG) accumulation is associated with impaired left ventricular diastolic function, independently of age, BMI, heart rate, visceral fat, and diastolic blood pressure (Rijzewijk et al., 2008; Ruberg, 2007). Myocardial TG accumulation is a crucial pathophysiological characteristic associated with several disorders such as metabolic syndrome, type 2 diabetes mellitus and obesity (Christoffersen et al., 2003; McGavock et al., 2007). In these pathologic situations, an excess supply of fatty acids (FA) leads to myocardial TG accumulation in the form of cardiomyocyte lipid droplets (LDs). However, the formation of LDs is not directly associated with heart dysfunction as LDs are also found in non-pathological conditions, such as the muscle of healthy athletes (Muoio, 2012). Indeed, the impact of myocardial LDs is different depending on the metabolic context. In exercise training, FA released from LDs are oxidized in the mitochondria to satisfy energy demand, whereas in sedentary subjects the FA overload promotes mitochondrial and endoplasmic reticulum stress, inflammation and lipid signaling alterations (Muoio, 2012). FAs are the main substrate for mitochondria-energy production in cardiomyocytes. Under hypoxia, the reduced availability of oxygen limits mitochondrial FA oxidation and favors the accumulation of cytoplasmic FAs, which are potential inducers of lipotoxicity. Hence, the adverse effects of myocardial LDs appear to be related to restricted mitochondrial FA β-oxidation rather than to LD accumulation. LD monolayer surface is composed of crucial proteins such as perilipins that determine LD interactions and dynamics (Bickel et al., 2009; Olofsson et al., 2008). In particular, Perilipin 5 (Plin5) is a LD protein that plays a key role in metabolic and physical inter-organelle interactions of LDs and mitochondria in the myocardium (Kuramoto et al., 2012; Wang et al., 2011). These authors proposed that Plin5 regulates the delivery of FA from LD to mitochondria, protecting the mitochondria against an excessive FA supply.

Cardiomyocytes have a rather limited capacity to store TG so the balance between FA uptake, storage and mitochondrial oxidation is tightly regulated (Taegtmeyer, 1994). Regarding uptake, two potential sources of FA reach the heart; circulating non-esterified FAs (NEFA) bound to plasma albumin, and circulating esterified FAs in the form of TG and cholesteryl esters (CE) transported by large TG-containing lipoproteins including remnant chylomicrons and very low density lipoproteins (VLDL) (Atkinson et al., 2003; Bharadwaj et al., 2010; Pillutla et al., 2005). Remarkably, increased plasma VLDL levels have been reportedly related with myocardial LD accumulation in insulin-resistance patients (Goldberg, 2001; Rijzewijk et al., 2008). In this group of patients, there is also an increase in plasma small, dense, electronegative LDL [LDL(−)] (Sanchez-Quesada et al., 2001, 2005; Zhang et al., 2005). LDL(−) has been defined as a pool of LDLs modified by different mechanisms presenting pro-inflammatory activity, reduced affinity to the LDL receptor and increased binding to arterial proteoglycans, among other properties (Sanchez-Quesada et al., 2004, 2012a,b). LDL(−) induces a plethora of deleterious effects on several cell types involved in atherogenesis including the release of cytokines by endothelial cells, monocytes and lymphocytes, the impairment of endothelial progenitor cell differentiation, endothelial apoptosis and platelet activation (Sanchez-Quesada et al., 2012a). Regarding the effect of LDL(−) on cardiomyocytes, only one study analyzed the induction of apoptosis indirectly by cytokines released from endothelial cells exposed to LDL(−) (Lee et al., 2012). Therefore, the direct impact of LDL(−) on cardiomyocytes remains unknown. The aim of this work was to assess the effect of LDL(−) on intracellular lipid content and Plin5 levels in cardiomyocytes and to determine whether these LDL(−) effects were influenced by hypoxia.

2. Materials and methods

2.1. HL-1 cell culture

The murine HL-1 cell line was generated by Dr. W.C. Claycomb (Louisiana State University Medical Centre, New Orleans, LA, USA) and kindly provided by Dr. U Rauch (Charité-Universitätmedizin Berlin). These cells showed cardiac characteristics similar to those of adult cardiomyocytes such as the presence of highly ordered myofibrils and cardiac-specific junctions in the form of intercalated disks as well as the presence of cardio-specific voltage dependent currents such as the I_{Kr} and an ultrastructure similar to primary cultures of adult atrial cardiac myocytes (Claycomb et al., 1998; White et al., 2004). The HL-1 cells were maintained in a Claycomb Medium (JRH Biosciences, Lenexa, KS, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen Corporation, Carlsbad, CA, USA), 100 μM norepinephrine, 100 units/mL penicillin, 100 μg/mL streptomycin, and L-glutamine 2 mM (Sigma Chemical Company, St. Louis, MO, USA) in plastic dishes, coated with 12.5 μg/mL fibronectin and 0.02% gelatin, in a 5% CO_2 atmosphere at 37 °C.

2.2. LDLs isolation, modification and characterization

Total LDL was isolated by sequential ultracentrifugation using KBr gradients, in the density range 1.019–1.063 g/mL, and then subfractionated into native LDL (LDL(+) and LDL(−) by anion-exchange chromatography, as described (Sanchez-Quesada et al., 2003). LDL(+) and LDL(−) were isolated from 10 different pools of plasma. Characterization of LDL subfractions included lipid (esterified and free cholesterol, triglycerides, phospholipids, NEFA) and apolipoprotein B-100 (apoB) content. These components were quantified by commercial standardized methods (Roche Diagnostics, Switzerland; and Wako Chemicals, Germany) adapted to a Cobas c501 autoanalyzer. Charge-dependent electrophoretic mobility was analyzed by 0.5% agarose gel electrophoresis. ApoB integrity was tested by SDS-PAGE in 10% acrylamide gels. The degree of aggregation was assessed by non-denaturing polyacrylamide gradient gel electrophoresis (GGE), as previously reported (Sanchez-Quesada et al., 2002). Lipoperoxidation was estimated by calculating the ratio at 205/234 nm of the phosphatidylcholine peak area separated by normal phase HPLC, as described (Bancells et al., 2008). After incubation with HL-1 cardiomyocytes, lipid and protein composition of the remaining LDLs re-isolated from the culture medium was determined again, as stated above.

NEFA enriched LDL (NEFA-LDL(+)) was obtained by incubation of LDL(+) (0.5 g/L of apoB) with a mixture of NEFA 2 mM (palmitic/oleic/linoleic acids in proportion 35:20:45) for 4 h at 37 °C, as described (Benitez et al., 2004). PLA2-treated LDL (PLA2-LDL(+)) was obtained by incubation of total LDL (1 g/L of apoB) with purified secretory PLA2 (5 ng/mL) for 2 h at 37 °C as described (Benitez et al., 2004). Modified LDLs were filtered through 0.22 μm, and concentrated by ultracentrifugation. GGE was performed to confirm increased negative electric charge (not shown). All preparations of NEFA-LDL(+) or PLA2-LDL(+) had a NEFA content ranging from 130 to195 mol NEFA/mol apoB. LDL is expressed as mg apoB/mL throughout the text.

2.3. DiI-labeling of LDL(+) and LDL(−)

LDL(+) and LDL(−) were labeled with 1,1′-dioctadecyl-3,3,3′,3′ tetramethylindocarbocyanine (DiI) by incubating LDL (1 mg/mL) in PBS–0.5% BSA with 100 μL of DiI in DMSO (3 mg/mL) for 8 h at 37 °C. The density of the LDL solution was adjusted to 1.063 g/mL, and LDL particles were re-isolated by ultracentrifugation, dialyzed, and

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