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Adverse interaction between HDL and the mass of myocardial infarction

Alexandre A.S. Soares[a,b], Luiz Sergio F. Carvalho[a,b], Isabella Bonilha[a,b], Vitor W. Virginio[a,b], Wilson Nadruz Junior[a], Otavio Rizzi Coelho-Filho[a], Jose C. Quinaglia e Silva[c], Orlando Petrucci Junior[d], Andrei C. Sposito[a,b,*], on behalf of Brasilia Heart Study Group

[a] Cardiology Division, State University of Campinas, Campinas, São Paulo, Brazil
[b] Laboratory of Atherosclerosis and Vascular Biology, State University of Campinas, Campinas, São Paulo, Brazil
[c] Hospital de Base do Distrito Federal, Brasilia, Distrito Federal, Brazil
[d] Cardiac Surgery Division, State University of Campinas, Campinas, São Paulo, Brazil

HIGHLIGHTS

- Higher HDL-C levels on first day of ST-segment elevation MI predict larger MI mass.
- HDL-C predicts MI mass assessed by cardiac magnetic resonance or CKMB enzymatic curve.
- The interaction between HDL-C and MI mass depends on early (< 4 h) coronary reperfusion.
- Healthy HDL infused during reperfusion in an *ex vivo* animal model reduces MI size.
- HDL from MI patients fail to reduce MI size in the same model.

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ABSTRACT

Background and aims: Coronary reperfusion with HDL from healthy volunteers attenuates ischemia and reperfusion injury in animal models. In myocardial infarction (MI) patients, such an interaction is unclear. Hence, our first objective was to verify if there is interaction between HDL-C and MI mass in patients and the role of coronary reperfusion in the interaction. Furthermore, we investigated whether the effect in MI size of reperfusion with HDL obtained from healthy participants or MI patients could differ.

Methods: HDL-C was measured the first day after MI and MI mass was quantified by cardiac magnetic resonance (n = 94) and peak CKMB (n = 393). In an *ex vivo* rat heart model, we compared MI area and dP/dt max after coronary reperfusion with HDL from MI patients or healthy volunteers.

Results: HDL-C above the median (35 mg/dL) was associated with higher peak CKMB [255 (145–415) *vs.* 136 (84–287) UI/L; $p = 0.02$], higher MI mass [17 (9–21) *vs.* 10 (6–14) g; $p < 0.01$] and lower left ventricular ejection fraction [47 (34–53) *vs.* 51 (43–59); $p = 0.02$] than their counterparts. In restricted cubic spline and multivariate linear regression, HDL-C was directly associated with peak CKMB ($p < 0.01$) and MI mass ($p < 0.01$) only in reperfused patients with time to reperfusion < 4 h. Reperfusion with healthy HDL, but not from MI patients, reduced MI mass ($p < 0.01$) and improved dP/dt max ($p = 0.02$).

Conclusions: In MI patients undergoing early coronary reperfusion, HDL-C levels at admission are directly associated with MI size. In contrast to healthy HDL, reperfusion with HDL from MI patients do not reduce MI area in an *ex vivo* animal model.

1. Introduction

Early reperfusion stands as the main goal for patients presenting with ST segment elevation myocardial infarction (STEMI) and the reason for the substantial reduction in lethality in the last decades [1]. Still, mechanisms of cellular injury are triggered by reperfusion and may result in up to 50% of the final mass of myocardial infarction (MI), adversely affecting the prognosis of the patients [2]. Hence, attenuation of ischemia/reperfusion (I/R) injury has become the new frontier for further declines of short- and long-term mortality after STEMI.

* Corresponding author. Laboratory of Atherosclerosis and Vascular Biology (AteroLab) Cardiology Department, State University of Campinas (Unicamp), 13084-971, Campinas, SP, Brazil.
E-mail address: andreisposito@gmail.com (A.C. Sposito).

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Recently, coronary reperfusion with high-density lipoprotein (HDL) attenuated myocardial I/R injury reducing final MI size in *ex vivo* [3] and *in vivo* [4] animal models. However, this cardioprotective capacity of HDL could not be demonstrated in humans. HDL-cholesterol (HDL-C) levels is the most available measurement of HDL composition in clinical practice and is highly correlated with total HDL mass [5]. A first step in demonstrating a cardioprotective effect of HDL at the MI site would be the verification of a relationship between HDL-C and the infarcted mass. Only one previous study has focused on this possible association [6], with more studies exploring the association between plasma HDL-C levels and the clinical outcome after acute coronary syndromes. The results are conflicting [7–10], particularly in the STEMI population [8,11]. The duration of coronary occlusion and the extent of myocardial injury could justify the inconsistency in these findings [8,12]; prolonged occlusions resulting in extensive MI mass hypothetically minimize the protective role of HDL. However, in animal models even with permanent ligation of left descending coronary artery, higher HDL-C levels were still associated with smaller scars, better LV function and survival at long term [13]. Thus, we defined the investigation of the association between the mass of HDL and the infarcted mass in STEMI patients as the first objective of this study, and whether this interaction is influenced by the presence and time to coronary reperfusion.

Confirming in humans the animal model findings of a protective role of HDL in MI is also challenging because, in this condition, available HDL is exposed to the acute phase inflammatory response and, over a long period, to risk factors that preceded the coronary event. Indeed, HDL in patients who manifest MI is often dysfunctional, with reduced antioxidant and anti-inflammatory activities [14,15]. In addition, the acute inflammatory and oxidative response elicited to promote tissue repair after MI affects most of the HDL capabilities, such as lipid transfer [16], and antioxidant and anti-inflammatory actions [17]. The changes in HDL composition and structure not only attenuate HDL protective functions, but also can turn HDL into a dysfunctional pro-inflammatory particle [17]. Therefore, HDL particles from MI patients may interact differently with mechanisms of I/R injury, losing their cardioprotective properties during the acute phase of STEMI. To test this hypothesis, the second part of this study was designed to verify if the effect on the I/R lesion may differ when the infarcted site is reperfused with HDL from healthy volunteers or patients with MI.

2. Materials and methods

2.1. Study population and laboratory analyses

For this prospective study, data from consecutive patients with STEMI enrolled in the Brasilia Heart Study (BHS) were used. A more detailed description is published elsewhere [18]. Briefly, BHS inclusion criteria were as follows: (i) $<$ 24 h after the onset of MI symptoms, (ii) ST-segment elevation $\geq$ 1 mm (limb leads) or $\geq$ 2 mm (precordial leads) in two contiguous leads, (iii) myocardial necrosis, as evidenced by an increase in at least one value above the 99th percentile above the reference limit of CK-MB (25 U/L) and troponin I (0.04 ng/mL) followed by a decline of both. BHS proceedings were in accordance with the 1975 Helsinki Declaration and local Ethic Committee approved the study. Participants were only enrolled after signing informed consent. BHS is registered at ClinicalTrials.org (NCT02062554).

2.2. Clinical evaluation

Upon admission, all patients (n = 393) underwent detailed medical interview, physical examination, electrocardiogram and blood sampling for biochemical analysis. The investigators documented all treatments and procedures performed during hospitalization and did not interfere with the attending physicians' decisions. Diabetes mellitus (DM) was defined as the use of anti-diabetic medications, prior diagnosis of diabetes, or glycated hemoglobin (HbA1c) $\geq$6.5% at hospital admission. Hypertension was defined by the use of antihypertensive drugs or a previous diagnosis of hypertension. The classification proposed by Killip was used as part of initial risk stratification after STEMI [19]. Anthropometrical measurements obtained were body weight (kg), height (m), and waist circumference (WC).

2.3. Biochemical analysis

The following measures were determined thereupon admission (D1) and in the fifth day (D5) after MI: total cholesterol (CHOD-PAP, Roche Diagnostics, Mannheim, Germany), HDL cholesterol (HDL-C without pre-treatment sample, Roche Diagnostics, Mannheim, Germany), triglycerides (GPO-PAP, Roche Diagnostics, Mannheim, Germany), and C-reactive protein (CRP) (high-sensitivity CRP, Cardiophase, Dade Behring, Marburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. CKMB was measured with Immulite Automated Analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA) in blood sampling at admission and every 6 h from admission to normalization of its value. Glomerular filtration rate (GFR) was estimated by the abbreviated MDRD equation (mL/min): 186 x (creatinine/88.4)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742, if female) x (1.210, if black).

2.4. Cardiac magnetic resonance imaging (CMRi)

Consecutive patients who accepted to undergo CMRi (n = 94) were studied supine in a 1.5 T scanner (Signa CV/i, General Electric Healthcare, Milwaukee, WI, USA) 2 weeks after hospital discharge as described previously by our group [20]. CMRi data were analyzed by a consensus of two experienced cardiologists blinded to any clinical or laboratorial. Left ventricular (LV) myocardial mass was determined by tracing the epicardial and endocardial borders in matching short-axis cine locations at end-systole [21,22]. LV ejection fraction was measured by standard Simpson's rule using summation of short-axis locations. A previously described full-width half-maximum method [23] was applied to define the total MI mass using a semiautomatic detection algorithm based on the signal intensity (SI) of the remote normal myocardium and the infarct myocardium. Infarcted mass is shown in grams. Previous MI areas were excluded of the calculations of the infarct mass.

2.5. Ex vivo reperfusion model

Male adult Wistar rats (n = 22), weighing 300–400 g, were provided by the Centro Multidisciplinar para Investigação Biológica na Área da Ciência em Animais de Laboratório (CEMIB/UNICAMP). Rats were maintained in a 12-h light, 12-h dark cycle, constant temperature (23 ± 2 °C), circulating air, and for all experimental conditions free access to water. The protocol approved by Ethics Committee on Animal Experimentation of the State University of Campinas (UNICAMP) (4418-1/2016). All animals received care according to the recommendations of the Committee on Care and Use of Laboratory Animals - Institute of Laboratory Animal Resources (ILAR) - National Research Council, United States. For HDL infusion preparation, plasma was collected from healthy volunteers and from STEMI patients (n = 3) at D1 and D5. The healthy male volunteers (n = 11, 23 ± 3 years old) provided informed consent before blood sampling.

HDL was isolated by gradient ultracentrifugation and dialyzed against phosphate buffered saline (PBS) according to the method described by Chapman [24]. Rats were anaesthetized via an intraperitoneal injection of sodium thiopental (80 mg/kg body weight), followed by sodium heparin (3.000UI/kg body weight). The heart was quickly excised and the aorta cannulated for retrograde perfusion in a Langendorff apparatus. Isolated rats hearts were perfused with oxygenated Krebs Henseleit buffer under normothermy and constant pressure of 100 cm H_2O with gravitational method. A saline-filled balloon was inserted through the mitral valve into the left ventricular cavity,

connected to a pressure transducer to maintain a diastolic pressure between 5 and 10 mmHg and allow continuous monitoring of left ventricular pressure. Isolated rats hearts were subject to a 10 min stabilization period followed by 35 min regional ischemia then a 90 min reperfusion period. The regional ischemia was achieved by ligation of the left anterior descending coronary artery.

In the first 7 min of reperfusion, a total of 7 mL of HDL from healthy volunteers and from STEMI subjects (D1 and D5) was infused via a side pump adjusted to a specified concentration (200 μg protein/mL), as previously described [25]. Controls were infused with PBS buffer (n = 5). Left-ventricular systolic and end-diastolic pressures, as well as the maximum rate of change in LV pressure (dP/dt), a measure of inotropic state, were continuously recorded. The percentage of dP/dt recovery 30 min after ischemia compared to baseline was used to compare groups. At the end of reperfusion, the left anterior descending coronary artery was occluded for infusion with Evans blue dye (0.25%) to determine the area of the non-myocardial infarction. Then, the hearts were withdrawn from the apparatus, sliced and stained with triphenyltetrazolium chloride. Infarct size was evaluated by the analysis of the dead cells area normalized to the risk area using computerized planimetry (Digimizer, MedCalc Software, Ostend, Belgium).

2.6. Statistical analysis

Data are presented as mean ± standard deviation for variables with normal distribution and as median (interquartile range) for skewed variables. Correlation analyses were performed using the Pearson test for normally distributed variables or Spearman for skewed variables. Patients were divided into two groups above and below the median HDL-C levels and compared with using Student t-test and Mann-Whitney test when appropriated. Categorical variables were compared using Fisher's exact test. A logarithmic transformation of peak CKMB was performed to perform an analysis of covariance (ANCOVA) to assess the association between HDL-C groups and peak CKMB. Adjustments for age, gender, LDL-C and triglycerides levels were made. Restricted cubic spline models was used to assess linear and potentially nonlinear association between plasma HDL-C levels and myocardial infarcted mass. The association was also assessed by linear regression in an unadjusted analysis (model 1) and models progressively adjusted for age, gender, age, gender, LDL-C, triglycerides, hypertension and smoking (models 2 to 4). Patients who underwent coronary reperfusion were segregated into five subgroups according time to reperfusion: < 2 h (n = 11), 2–4 h (n = 26), 4–6 h (n = 14), 6–8 h (n = 14) and ≥ 8 h (n = 10). Simple linear regression was performed in each subgroup to assess the influence of time to reperfusion in the association between MI mass by CMRi and HDL-C. A third order polynomial least squares surface fit was generated with Surface Fitting Tool of Matlab (Mathworks, Natick, MA, USA) to visually explore the association peak CKMB with HDL-C and time to reperfusion. Mann-Whitney test was used to compare the groups in animal study. Statistical significance was defined as $p < 0.05$. Analyses were performed using IBM SPSS Statistics 20.0 (IBM, Armonk, NY, USA) and SAS 9.2 (SAS Institute, Cary, NC, USA) for MAC.

3. Results

3.1. Demographic data

Baseline characteristics of participants are shown in Table 1. The population was predominantly male (79%), with a mean age of 60 years and a high burden of cardiovascular risk factors, reflecting a typical population presenting with STEMI. Clinical characteristics were not different between patients above or below median HDL-C levels. Lipid profile was significantly different between groups, as patients with higher HDL-C at admission showed also higher LDL-C and lower triglycerides levels. There was no significant difference in renal function, glucose metabolism or inflammatory markers between groups. In the univariate analysis shown in Table 1, median peak CKMB is significantly higher in the group above median HDL-C (255 [145–415] *vs.* 136 [84–287], $p = 0.02$). In multivariate analysis using ANCOVA adjusted by age, gender, LDL-C and triglycerides, the relationship remained significant ($p = 0.04$).

Table 1
Clinical, laboratorial and CMRi patients below and above median HDL-C levels (35 mg/dL) characteristics.

Clinical characteristics	HDL-C ≤ 35 mg/dL (n = 50)	HDL-C > 35 mg/dL (n = 44)	*p*
Age, years	59 ± 7	62 ± 10	0.07
Male, n (%)	39 (78)	35 (79.5)	1.00
Diabetes mellitus, n (%)	22 (44)	13 (29.5)	0.20
Hypertension, n (%)	37 (74)	25 (56.8)	0.09
Previous statin use, n (%)	19 (38)	15 (34.1)	0.83
Previous MI, n (%)	5 (10)	3 (6.8)	0.72
Previous angioplasty, n (%)	3 (6)	1 (2)	0.62
Previous CABG, n (%)	1 (2)	0 (0)	1.00
Previous stroke, n (%)	1 (2)	3 (6.8)	0.34
Current smoking, n (%)	23 (46.9)	12 (27.3)	0.06
Anterior MI, n (%)	15 (30)	20 (46.5)	0.14
Killip > 1, n (%)	2 (4.4)	4 (9.1)	0.46
Systolic blood pressure, mmHg	138 ± 30	147 ± 34	0.16
Diastolic blood pressure, mmHg	86 ± 20	91 ± 20	0.27
Heart rate, bpm	73 ± 14	79 ± 17	0.10
Body Mass Index, kg/m^2	27.8 ± 3.8	27.3 ± 4.1	0.55
Abdominal circumference, cm	98 ± 11	97 ± 11	0.70
Laboratorial characteristics			
LDL-C, mg/dL	119 ± 34	139 ± 45	**0.02**
Triglycerides, mg/dL	194 (105–288)	110 (72–165)	< **0.01**
CKMB peak, U/L	136 (84–287)	255 (145–415)	**0.02**
Glycaemia, mg/dL	162 ± 75	143 ± 52	0.17
HbA1c, %	6.1 (5.6–7.0)	6.0 (5.8–6.5)	0.77
CRP D1, mg/L	0.6 (0.3–1.3)	0.8 (0.3–1.4)	0.72
CRP D5, mg/L	2.2 (1.2–6.9)	2.5 (1.7–4.9)	0.81
Glomerular filtration rate, mL/min	70 ± 22	70 ± 18	0.95
CMRi characteristics			
LV ejection fraction, %	51 (43–59)	47 (34–53)	< **0.01**
LV end diastolic final volume, mL	123 (110–135)	141 (116–177)	**0.02**
LV end systolic final volume, mL	59 (47–71)	72 (57–112)	**0.02**
LA diastolic volume, mL	62 (51–72)	65 (52–84)	0.28
LV mass, g	120 (105–141)	120 (109–135)	0.98
MI mass, g	10 (6–14)	17 (9–21)	< **0.01**
MI mass, % of LV mass	9 (5–12)	13 (8–18)	< **0.01**

Variables are expressed as mean ± standard deviation (normally distributed), median (interquartile range) (skewed distributed) or percent (categorical).
CABG: coronary artery bypass grafting; CKMB: creatine kinase-MB; CRP: C-reactive protein; D1: on the 1st day after MI; D5: on the 5th day after MI; HbA1c: glycosylated hemoglobin; HDL-C: high density lipoprotein cholesterol; LDL: low density lipoprotein cholesterol; MI: myocardial infarction LA: left atrium; LV: left ventricular.

Coronary reperfusion was achieved in 80% of patients, mostly through chemical thrombolysis and no significant difference was found between groups above or below median HDL-C levels. Reperfusion was confirmed by coronary angiography in all enrolled patients. The time from symptoms to reperfusion, use of main pharmacological therapies and in-hospital revascularization procedures were also similar (Supplemental Table 2).

3.2. Association between HDL-C and MI mass by CMRi

STEMI patients with HDL-C above the median (35 mg/dL) at admission had larger MI considering both absolute MI mass and relative to total LV mass as compared with those presenting with HDL-C below the median (Table 1). Accordingly, the group with higher HDL-C showed worse LV ejection fraction, LV end diastolic and end systolic volumes. HDL-C levels positively correlated with MI mass ($r = 0.307$; $p < 0.01$). To further verify linear or nonlinear association between HDL-C and MI

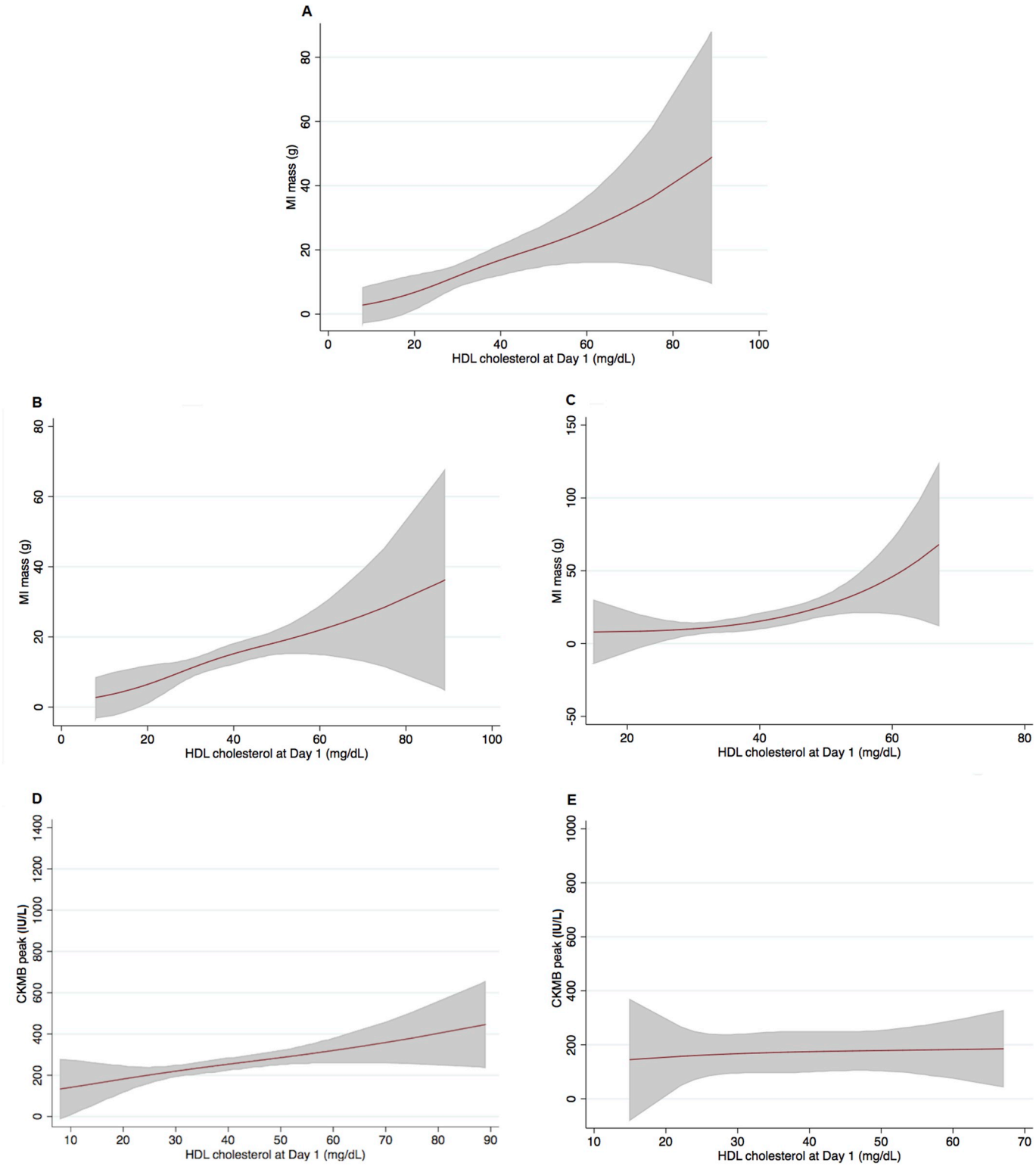

Fig. 1. Spline graphs showing associations between HDL cholesterol at Day 1 MI and infarct size according to status of reperfusion.
(A) Restricted cubic spline graphs showing (fitted curve with 95% confidence interval) linearity between MI mass measured by cardiac magnetic resonance imaging and HDL cholesterol at Day 1 after MI in the whole sample (p for trend < 0.01), which persisted in patients undergoing coronary reperfusion (p for trend < 0.01) (B), but not in patients without coronary reperfusion (p for trend = 0.22) (C). HDL cholesterol at Day 1 is also directly associated with CKMB peak in reperfused patients (p for trend = 0.02) (D), but not in those who were not reperfused (p for trend = 0.32) (E). CKMB: creatine kinase-MB; HDL: high density lipoprotein; MI: myocardial infarction.

mass, a restricted cubic spline model shown in Fig. 1A was applied and confirmed a linear relationship (p for trend < 0.01). Hence, multivariable linear regression models were performed to confirm the association (Supplemental Table 3). In the unadjusted model (model 1) and after progressive adjustment for age, gender, LDL-C, triglycerides, hypertension, and smoking history (models 2 to 4), HDL-C at Day 1

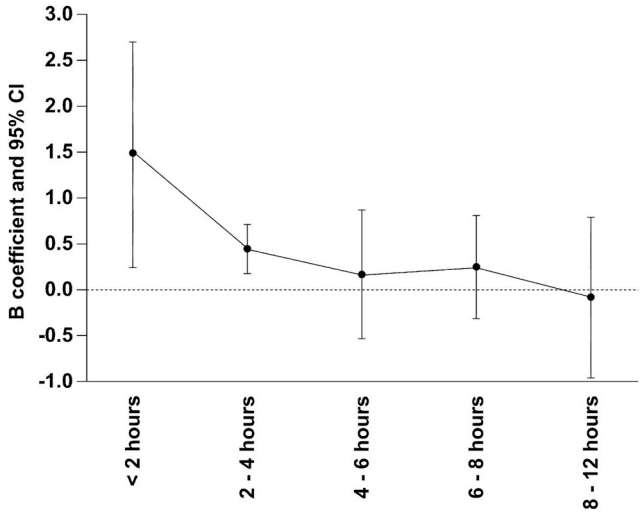

Fig. 2. Higher HDL-C levels predict higher MI mass by CMRi in STEMI patients reperfused until 4 h, but not after this period.

after MI remained associated with infarcted mass quantified by CMR.

Subsequently, patients undergoing (n = 75) or not (n = 19) coronary reperfusion were divided and models were repeated in each group. Both restricted cubic spline and linear regression models confirmed a direct association between HDL-C and MI mass in reperfused patients, but failed to find this relation in non reperfused ones (Fig. 1B and C and Supplemental Table 4).

Reperfused patients were further divided according to time to reperfusion in intervals of 2 h. The direct relationship between HDL-C and MI mass by CMRi was significant until 4 h to reperfusion, but the association was lost after this period. The increment of 1 mg/dL of HDL-C predicted approximately an increase of 1.5 g of MI mass measured by CMRi in the earliest reperfused subgroup (Fig. 2).

3.3. Association between HDL-C and peak CKMB

To further validate the results, data from the whole BHS cohort were used to amplify the results of the association between HDL-C and peak CKMB and the patients were also divided according to coronary reperfusion or not. HDL-C levels positively correlated with peak CKMB ($r = 0.119$; $p = 0.02$). Both restricted cubic spline model and the multivariable linear regression model adjusted by age, gender, LDL-C, triglycerides, hypertension, and smoking history demonstrated a positive association between HDL-C and peak CKMB in patients submitted to coronary reperfusion (n = 288), but no relation was found in patients who were not reperfused (n = 105) (Fig. 1D and E and Supplemental Table 5).

The association between peak CKMB with HDL-C and time to reperfusion is illustrated in Fig. 3. It is observed in the surface graph that the earlier the time to reperfusion the more inclined is the curve of peak CKMB along the HDL-C axis, which represents a stronger association between the two variables.

3.4. HDL from healthy and MI subjects infusion during reperfusion in isolated rat hearts

To verify the results in humans, Langendorff-perfused rat hearts were submitted to temporary ligation of left anterior descending coronary artery followed by 7-min reperfusion with PBS buffer (controls), HDL isolated from healthy volunteers or HDL from STEMI patients obtained at D1, D5. HDL from healthy donors reduced the infarcted area by up to 20% in comparison to controls ($p < 0.01$) and to HDL from MI patients in D1 and D5 ($p = 0.04$ and 0.02, respectively). Infarcted size of hearts that received HDL from MI patients was numerically larger than controls, but the difference was not statistically significant neither in D1 nor D5 (Fig. 4A). At 30 min of reperfusion, inotropism measured by dP/dt max was superior in hearts reperfused with healthy HDL than controls, but not different from those who received MI HDL infusion (Fig. 4B).

4. Discussion

The findings of the study revealed a positive linear association between HDL-C levels and infarcted mass in the selected population of STEMI patients undergoing coronary reperfusion. Both an imagining method, CMR, and a biochemical surrogate of MI size, CKMB, indicated the association. No such association was found in patients who could not achieve coronary reperfusion or undergoing late reperfusion, indicating a possible connection through mechanisms of I/R injury. Patients with higher HDL-C levels also presented worse LV ejection fraction and larger LV volumes at CMR. In an *ex vivo* animal model of MI, reperfusion with HDL from healthy volunteers reduced the infarcted area and resulted in better systolic function compared to controls, while the reperfusion with HDL from MI patients failed to show any improvement.

Only one previous study investigated the association between HDL-C and MI mass quantified by CMR, with no regard to reperfusion, and found a relationship in the opposite direction [6]. In this study, patients were segregated by HDL-C, those with values < 40 mg/dL also had higher levels of CRP and more frequently anterior wall MI. Since the inflammatory activity is strongly related to HDL dysfunction, it is possible that the disagreement is at least in part related to the clinical phenotype segregation induced by this dichotomization based on HDL-C levels [16]. Moreover, a higher proportion of anterior wall myocardial infarction would be a preponderant factor for MI size when compared to HDL levels [26]. In our study, we investigated the two components as continuous variables and used multivariate analysis to confirm an independent association. In addition, in our HDL-based dichotomized analysis, baseline CRP and the proportion of anterior wall MI were not different.

Two previous large (> 20,000 patients) studies found a positive association between HDL-C and plasma myocardial necrosis markers, CKMB [8] and troponin [11]. Both studies originated from the Korean Registry of Acute Myocardial Infarction (KAMIR), and found higher levels of myocardial necrosis markers (MNMs) in groups with higher levels of HDL-C. Such a large sample also precluded CMR for all patients, but previous studies consistently showed a good correlation between MNMs and MI size quantified by CMR [27,28]. Since all KAMIR patients underwent coronary reperfusion, the interaction with coronary reperfusion could not be verified. In response to our study first objective and in addition to the available evidence, our findings indicate that reperfusion is required to have an interaction between HDL and MI mass.

Our study explored, for the first time, the influence of time to reperfusion on the impact of HDL-C levels on MI mass. We found that after delays greater than 4 h to reperfusion, the interaction between HDL and MI mass is lost. This finding is also observed in other mediators capable of interacting with mechanisms of ischemia reperfusion injury such as adenosine. A subanalysis of the Acute Myocardial Infarction Study of Adenosine (AMSTAD-II) trial showed that adenosine infusion within the first 3.17 h onset of STEMI enhanced survival and reduced infarct size at high doses, but beyond that period failed to show benefits [29]. One probable explanation is that most of the damage in the MI area has already occurred as a result of long ischemia, leaving few cardiomyocytes still susceptible to mechanisms of injury by reperfusion. The wide confidence interval of the linear regression between HDL-C and MI mass in the subgroup of patients reperfused in the first 2 h from MI symptoms onset can be either a consequence of patients' heterogeneity in terms of I/R injury vulnerability or the difference between the time from symptoms onset and the real time of

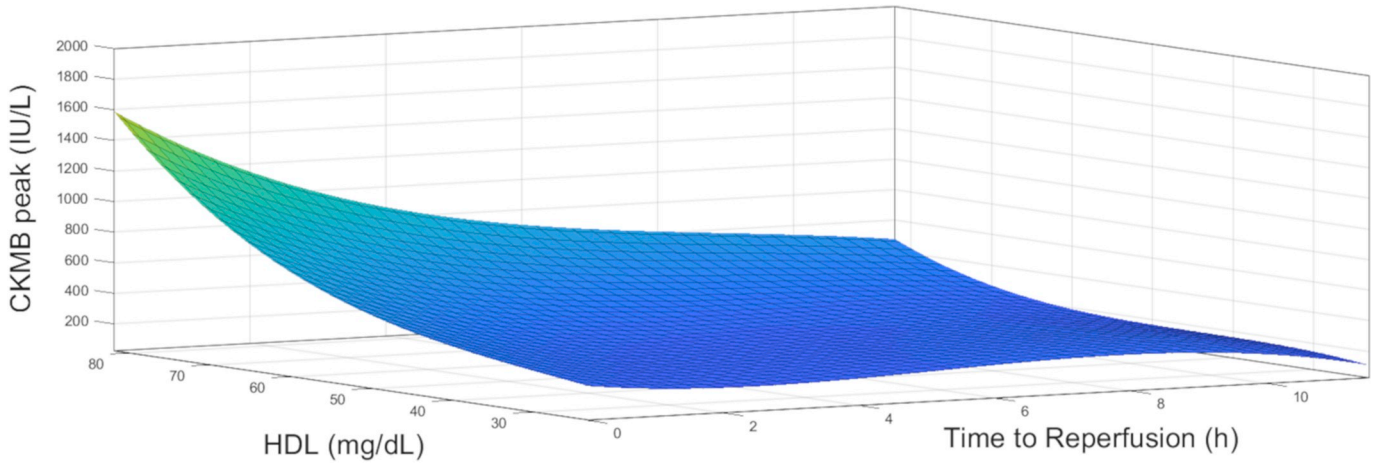

Fig. 3. Stronger association between peak CKMB and HDL-C in lower time to reperfusion after MI.
Third ordem polynomial least squares surface fits for the association of peak CKMB with HDL-C and time to reperfusion. CMRi: cardiac magnetic resonance imaging; MI: myocardial infarction; STEMI; HDL-C: high-density lipoprotein cholesterol.

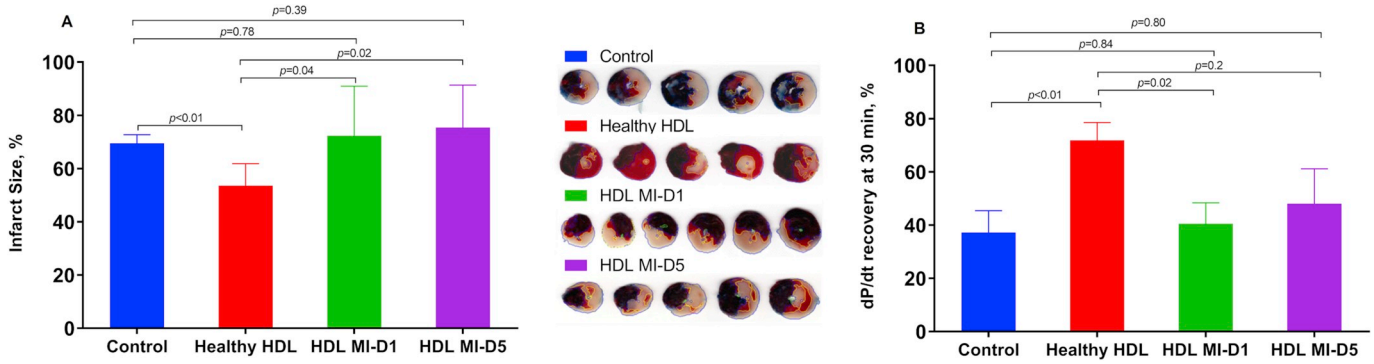

Fig. 4. HDL from healthy donors, but not HDL from MI patients infused during reperfusion reduces infarct size and improves contractility recovery after MI.
(A) Langendorff-perfused rat hearts were subject to 35 min of regional ischemia through temporary ligation of the left anterior descending coronary artery, and during reperfusion received 7 min infusion of PBS (Controls, n = 5), HDL (200 μg/mL) from healthy volunteers (n = 11), HDL from myocardial infarction patients collected at D1 (n = 3) and D5 (n = 3). Infarct size was determined as dead cells area normalized to the risk area using computerized planimetry. The infarct size was significantly reduced after infusion of healthy HDL compared to controls, HDL MI-D1 and HDL MI-D5. (B) To evaluate inotropism after ischemia, the maximum rate of change in left ventricular pressure (dP/dt máx) recovery after 30 min of reperfusion was compared with baseline. The dP/dt máx recovery was also better after the infusion of healthy HDL than in the other groups. Data are median ± confidence interval 95%. D1: the first day after MI; D5: the fifth day after MI; dP/dt máx: the maximum rate of change in left ventricular pressure; HDL: high-density lipoprotein cholesterol; MI: myocardial infarction.

ischemia. In addition, in this time frame, the modifications of HDL by MI are still minor [17] and the functional and phenotypic characteristics of HDL would reflect the patients' diversity in cardiovascular risk factors background before the acute coronary event.

The protective function of HDL against I/R injury was previously demonstrated with healthy HDL infusion after MI induction [3,4]. Thus, this infused HDL was free from the chronic inflammatory phenotype typical of high-risk patients, and from the intravascular remodeling induced by the inflammatory and oxidative response elicited by MI. Our study confirmed that infusion of HDL from healthy volunteers attenuated I/R injury, but infusion of dysfunctional HDL obtained from MI patients was ineffective. HDL can reduce the MI mass by a broad spectrum of mechanisms, including antioxidant activity, increased glucose uptake by the cardiomyocyte [30], and inhibition of I/R injury by apolipoprotein M, sphingolipid sphingosine 1 phosphate (S1P) [31] or apolipoprotein J [32,33]. In contrast, increasing the content of apolipoprotein C-III (apoC-III) in HDL may even promote apoptosis during acute coronary syndromes. ApoC-III can stimulate proapoptotic signaling by phosphorylation of p38-MAPK and upregulation of the endothelial expression of proapoptotic tBid [32]. In addition, HDL from acute coronary syndrome patients can induce the inhibitory Thr495 phosphorylation of endothelial nitric oxide synthase (eNOS) reducing endothelial nitric oxide production, which in turn increases nuclear translocation and DNA biding of NF-kB, a proinflammatory transcription factor involved in the upregulation of leukocyte adhesion molecules [14]. The migration of leukocytes cells into the infarct area, where they mediate cardiomyocyte death by vascular plugging, releasing degradative enzymes, and generating reactive oxygen species, is one of the mechanisms of I/R injury [2].

Thus, the contribution of HDL to the size of MI mass may vary in both directions: from reduction to increase. Our findings in humans suggest a harmful role of HDL from MI patients in the infarcted mass, as opposed to the neutral effect found in the animal model. The inherent limitations of isolated rat heart model, such as the lack of the humoral background found after MI, the possible preconditioning during the excision and the use of hearts free from cardiovascular risk factors could explain the divergent results. In addition, HDL-C concentration is unrelated to the particles quality, but previous studies of healthy HDL infusion show a dose-dependent effect of HDL on I/R injury [3], so a higher concentration of HDL from MI patients could be necessary to negatively impact the infarcted area as our findings in humans suggest. Moreover, we cannot rule out typo II error due to the small sample size and the heterogeneity of the characteristics of HDL from MI patients.

Recently, the infusion of a phosphatidylcholine-rich reconstituted

HDL (rHDL), the CSL-112, in patients with a recent MI, increased cholesterol efflux capacity of HDL [34]. Although many other features of HDL seem to be more relevant to prevent I/R injury than cholesterol efflux, this potential benefit provided plausibility for running the 'ApoA-I Event reducinG in Ischemic Syndromes II' (AEGIS-II) clinical trial with 17,000 patients with acute coronary syndrome. In this assay, 6 g of rHDL will be injected on top of about 20 g of native HDL available in the bloodstream of these patients during the acute phase of coronary event. Our present findings raised concerns about the possibility of unconstructive impact that may result from a negative finding in the AEGIS-II clinical trial on the development of HDL-based therapy for I/R injury. The 30% dilution ratio between rHDL and native dysfunctional HDL in MI patients in this study may fall short to protect endothelial cells and cardiomyocytes and may add pessimism to a large list of negative trials with HDL-based therapies. Timing of rHDL infusion is a key point to be considered as at least mechanisms of I/R injury seem to be susceptible to modification by HDL only after brief periods of ischemia. Our findings also suggest that one possible path to HDL-targeted therapies in acute coronary syndromes could be a focus on HDL particles more resilient to the modifications triggered by post MI background, achieved by either the development of rHDL or modification of native HDL.

Studies designed to identify ideal phenotypic and functional characteristics of HDL to attenuate I/R injury are fundamental to safely delineate a proof-of-concept study. Our study did not have this purpose but demonstrated in an *ex vivo* animal model that HDL extracted from MI patients do not beneficially act as HDL from healthy individuals in the I/R lesion and may even act deleteriously. Nevertheless, while we have attempted to minimize confounders with multivariate analyzes, they may still be present due to the observational nature of our study and thus other sources of evidence need to be explored. Due to the unpredictability of MI, the absence of data about HDL characteristics prior to the event makes difficult to define if the association found between HDL-C and MI size is cause or consequence of MI. Furthermore, although HDL-C levels is highly correlated with HDL total mass [5], cholesterol is only one component of HDL particle. Thus, we need to acknowledge the fact that the main strength of our study is the provocative nature of a possible adverse role of HDL during MI. However, the lack of a broad characterization of HDL composition that could produce mechanistic insight precludes a conclusion of causal relationship between HDL-C levels and MI size.

In conclusion, our present results show that in STEMI patients submitted to coronary reperfusion higher levels of HDL-C at admission predict larger MI mass assessed by CMR imaging and CKMB. This association does not exist in the absence of timely coronary reperfusion. In addition, although healthy HDL infused during reperfusion in an *ex vivo* animal model of MI reduces infarcted area and attenuates systolic dysfunction, the infusion of HDL from MI patients fails to change these outcomes.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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Author contributions

AASS and ACS conceptualized and designed the study. AASS, LSFC and JCQS collected data and enrolled patients. VWV and IB were responsible for animal experiments. ORCJ was responsible for cardiac magnetic resonance analyses. AASS, LSFC, WNJ and ACS analyzed and interpreted the data. AASS and ACS drafted and wrote the manuscript. WNJ, OP, JCQS and ACS reviewed the manuscript. All authors had full access to all of data (including statistical reports and tables) in the study and can take responsibility for the integrity of the data and the accuracy of the data analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2018.12.002.

B coefficients and 95% confidence intervals of simple linear regression between MI mass quantified by CMRi and HDL-C at Day 1 after MI performed in each subgroup of reperfused patients divided into five subgroups according to time to reperfusion: < 2 h (n = 11), 2–4 h (n = 26), 4–6 h (n = 14), 6–8 h (n = 14) and ≥ 8 h (n = 10). CMRi: cardiac magnetic resonance imaging; MI: myocardial infarction; STEMI; HDL-C: high density lipoprotein cholesterol; ST-segment elevation MI.

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