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Peri-procedural tight glycemic control during early percutaneous coronary intervention up-regulates endothelial progenitor cell level and differentiation during acute ST-elevation myocardial infarction: Effects on myocardial salvage☆☆

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ABSTRACT

Background: We examined the effects of peri-procedural intensive glycemic control during early percutaneous coronary intervention (PCI) on the number and differentiation of endothelial progenitor cells (EPCs) and myocardial salvage (MS) in hyperglycemic patients with first ST-elevation myocardial infarction (STEMI).
Methods and results: We conducted a randomized, prospective, open label study on 194 patients with STEMI undergoing PCI: 88 normoglycemic patients (glucose < 140 mg/dl) served as the control group. Hyperglycemic patients (glucose ≥ 140 mg/dl) were randomized to intensive glycemic control (IGC) for almost 24 h after PCI (n = 54; 80–140 mg/dl) or conventional glycemic control (CGC, n = 52; 180–200 mg/dl). EPC number, differentiation, and SIRT1expression were assessed immediately before, 24 h, 7, 30 and 180 days after PCI. The primary end point of the study was salvage index, measured as the proportion of initial perfusion defect (acute technetium-99m sestamibi scintigraphy, performed 5 to 7 days after STEMI) and myocardium salvaged by therapy (6 months after STEMI). Hyperglycemic patients had lower EPC number and differentiation and lower SIRT1 levels than normoglycemic patients ($P < 0.01$). After the insulin infusion, mean plasma glucose during peri-procedural period was greater in CGC group than in IGC group ($P < 0.001$). The EPC number, their capability to differentiate, and SIRT1 levels were significantly higher in IGC group than in CGC, peaking after 24 h ($P < 0.01$). In the IGC group, the salvage index was greater than in patients treated with CGC ($P < 0.001$)

1. Introduction

A positive association between hyperglycemia at the time of the event and subsequent mortality from acute myocardial infarction (AMI) has been reported [1]. Moreover, a recent study suggests that stress hyperglycemia affects myocardial salvage, indicating that the manipulation of glucose levels could be a potential therapeutic target for salvaging ischemic damage [2]. Although the mechanisms underlying this association are not fully understood, evidence that the use of insulin to lower glucose concentrations decreases mortality in diabetic patients who have AMI [3] suggests that hyperglycemia is not simply an epiphenomenon of a stress response. Consequently, hyperglycemia at the time

Abbreviations: AMI, acute myocardial infarction; CGC, conventional glycemic control; EPCs, endothelial progenitor cells; IGC, intensive glycemic control; PBMC, peripheral blood mononuclear cells; MS, myocardial salvage; PCI, percutaneous coronary intervention; SIRT1, silent information regulator 1; STEMI, ST-segment elevation myocardial infarction.

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of AMI may be an important and potentially modifiable risk factor for poor outcome [4].

A growing body of evidence suggests that circulating endothelial progenitor cells (EPCs) are mobilized endogenously in response to tissue ischemia and, thereby, augment neovascularization of ischemic tissues [5,6]. In patients with AMI, EPCs, defined as $CD34^+/KDR^+$ progenitor cells, are increased in peripheral blood and are rapidly recruited to myocardium mediating a protective effect on vascular healing and ischemic preconditioning and salvaging the ischemic myocardium in the acute phase of AMI [7]. The impaired abundance and activation of EPCs at the time of myocardial infarction are known to occur in diabetic patients [8], although the mechanisms by which the diabetic status impairs EPCs mobilization and subsequent domiciliation to infarcted tissue are poorly understood. Our recent findings show that the number of EPCs was significantly higher in patients with good glycemic control than those with poor glycemic control [9], and when more glucose was added to EPC cultures in vitro, viability and proliferation decreased whereas apoptosis increased [9]. However, the role of hyperglycemia on dynamics of EPC mobilization in the acute coronary syndrome, in human, has not been investigated before. It has been suggested that hyperglycemia-induced overproduction of oxidative stress, through the acceleration of EPC senescence, can explain the EPC impairments observed in diabetes [10]. Indeed, in vitro studies demonstrated that in EPCs the levels and activity of mammalian silent information regulator 1 (SIRT1), implicated in the prevention of stress-induced senescence endothelial dysfunction [11], are downregulated during short-term exposure to high-glucose concentrations. Whether EPC levels are decreased and contribute to the poor outcome of AMI in hyperglycemic patients is not known, as well as it is unclear whether strict glycemic control, in the context of PCI, improves both the EPC mobilization and function. On this basis, our study evaluated whether the EPC number and activity during ST-segment elevation myocardial infarction (STEMI) is influenced by hyperglycemia as well as whether peri-procedural tight glycemic control during angioplasty revascularization for STEMI is capable of increasing EPC number and activity and potentially contributing to improved recovery from myocardial damage. Myocardial salvage is the principal mechanism by which patients with AMI benefit from reperfusion therapies [12]. It can reliably be quantified by 99mTc sestamibi imaging [12]. Repeated myocardial imaging with 99mTc-sestamibi performed early after symptom onset and 180 days after primary reperfusion treatment allows reliable assessment of the area at risk, final infarct size, and salvage index or the proportion of area at risk that is salvaged by reperfusion therapy [12]. However, no information is available on the effects of intensive glycemic control on myocardial salvage in patients with AMI. We undertook this study to investigate the value of myocardial salvage in patients with AMI treated with intensive glycemic control and the possible relationships with the functional characteristics of mobilized EPCs in the peripheral circulation.

2. Methods

2.1. Patients

This was a randomized, prospective, open label study to compare two therapeutic strategies after PCI with stent: intensive glycemic control vs. conventional glycemic control in patients with hyperglycemia and STEMI. In the simple randomization process we used computer generated random list. According to the recent statement by the American Heart Association, hyperglycemia was defined as an admission plasma glucose level of >140 mg/dl [4]. The control group included patients with a normal plasma glucose (<140 mg/dl). We analyzed the consecutive patients presenting with first STEMI admitted to the Department of Cardiology of the Cardarelli Hospital in Naples, Pineta Grande Hospital, Caserta and Evalgelico Hospital Villa Betania, Naples (Italy) from December 2009 to December 2012. Inclusion criteria included: age of 18 years or greater, presentation to the cardiac catheterization laboratory for PCI in the setting of first STEMI. All STEMI patients were referred to the cardiac catheterization laboratory within 12 h of presentation. Patients with left ventricular ejection fraction less than 25%, with previous myocardial infarction or previous PCI or/and coronary by-pass grafting were excluded. Moreover, patients with restenosis at 6 month follow-up were also excluded from the evaluations. The following patients were referred for urgent invasive diagnostics with the intention of performing PCI: symptom duration of 12 h or less and ST-segment elevation of 0.1 mV or greater in at least 2 contiguous leads (≥0.2 mV in V1–V3) or presumed new-onset left bundle-branch block. They were included in the study after they gave written informed consent. Patients who experienced unsuccessful fibrinolytic therapy were also referred for PCI. Routine analyses were obtained on admission before coronary angiography and before full medical therapy were started. A group of 15 healthy volunteers (33 ± 8 years) recruited at the clinic centers were used as healthy subjects for the evaluations of EPC number, differentiation, and SIRT1 normal values. The investigation conforms with the principles outlined in the Declaration of Helsinki for the use of human tissue or subjects. The Institutional Review Board approved the protocol.

2.2. Study protocol

Upon emergency wards admission, all patients were assigned to undergo prompt coronary revascularization. After PCI procedures, hyperglycemic patients were randomly assigned to intensive glucose control (IGC group) or to conventional glucose control (CGC group). Operators assigned the patients either in IGC or CGC groups according to a computer generated randomization list. Participants were randomly assigned following simple randomization procedures (computerized random numbers) to 1 of 2 treatment groups. In the CGC group, continuous insulin infusion of 50 IU Actrapid HM (Novo-Nordisk) in 50 ml NaCl (0.9% using a Perfusor-FM-pump) was started only when plasma glucose levels exceeded 200 mg/dl and adjusted to keep plasma glucose between 180 and 200 mg/dl. When plasma glucose fell <180 mg/dl, insulin infusion was tapered and eventually stopped. In the IGC group, insulin infusion started when plasma glucose levels exceeded 140 mg/dl and adjusted to maintain glycemia at 80–140 mg/dl. After the start of insulin infusion protocol, a glycemic control was provided every hour in order to obtain three consecutive values that were within the goal range. The infusion lasted until stable glycemic goal (ICG group: 80–140 mg/dl; CGC group: 180–200 mg/dl) for at least 24 h. After that glycemic goal was maintained for 24 h, the infusion was stopped and subcutaneous insulin was initiated. Insulin was given as short-acting insulin before meals and long-acting insulin (insulin glargine) in the evening throughout the period of hospital stay in ICG group. CGC patients received insulin to achieve a fasting and postprandial blood glucose <200 mg/dl. After discharge from the hospital, all patients with established diabetes and newly diagnosed diabetes were managed and followed for 30 days after PCI, as outpatients, to maintain HbA1c level at <7%, blood glucose level of 90–140 mg/dl and post-prandial blood glucose level of <180 mg/dl. With regard to the full medical therapy, the protocol stated that the use of concomitant treatment should be as uniform as possible and accorded to evidence-based international guidelines for STEMI [13].

2.3. Angioplasty procedure

Balloon angioplasty was performed according to standard techniques as recently described [14]. Dilatation of a stenotic vessel was considered successful if the residual stenosis of the lumen diameter was less than 30%. The analyses of all angiographic data were performed by operators who were unaware of the study groups to which the patients were assigned (Toshiba, Infinix CS-i).

2.4. Scintigraphic evaluation

At 5 to 7 days after STEMI, patients received an intravenous injection of 27 mCi (1000 MBq) of technetium Tc-99m sestamibi. Single photon emission computed tomography was performed within 6 to 8 h after the injection of the radioactive agent. A follow-up scintigraphy was performed 180 days after the reperfusion therapy. The defect size was quantified by using a 50% threshold. All measurements were performed in the scintigraphic core laboratory by operators who were blinded to treatment allocation. Quantitative measurements included the initial perfusion defect size (from the initial scintigram), final infarct size (final perfusion defect from the follow-up scintigram), degree of myocardial salvage (difference between initial and final perfusion defects), and salvage index (ratio between the degree of myocardial salvage and the initial perfusion defect size). Paired scintigraphic examinations were needed to obtain salvage index, which was the primary end point of the study.

2.5. EPC phenotypic characterization and quantification

Immediately before PCI and after insulin infusion (24 h) as well as at 7, 30, and 180 days after PCI, peripheral heparinized blood samples (15 ml) were collected in a fasting condition between 7:00 AM and 9:00 AM. EPC number ($CD34^+/KDR^+$) was determined on 100 μL of peripheral heparinized blood samples by flow cytometry analysis (FACScalibur instrument, BD Biosciences) as described [9,15] within 2 h from blood collection. Antibodies used were CD34 (FITC; Miltenyi Biotech) and KDR (PE; R&D System, FAB 357P). Collected heparinized blood samples were used to isolate EPC from peripheral blood mononuclear cells (PBMCs) as previously described [9,15]. Another aliquot of PBMCs was cultured for 7 days in order to evaluate cell differentiation capacity.

2.6. Evaluation of EPC differentiation

To determine the rate of EPC differentiation after 7 days of cell culture, the expression of endothelial markers, CD31 and KDR, was evaluated by FACS analysis and confocal laser-scanning microscopy. FACS analysis was performed as previously described [9,15] using CD31 (FITC; Biolegend) and KDR (PE; R&D System, FAB 357P) antibodies.

2.7. Cell culture and characterization of progenitor cells

To determine the functional capacity of circulating EPCs, we cultured MNCs for 7 days to quantify CD31, VEGFR2. MNCs were isolated from 20 mL of peripheral blood by a density gradient centrifugation method.18 MNCs were cultured on 2% gelatin/fibronectin-coated dishes at 106 cells/cm^2 as described previously [9,15]. Cells were cultured in M199 supplemented with 20% fetal bovine serum (Invitrogen/GIBCO, Carlsbad, Calif), 2 mmol/L glutamine, 100 U/mL penicillin, 100 U/mL streptomycin, and bovine pituitary extract (Invitrogen/GIBCO). At 7 days of culture, to visualize the upregulation of endothelial markers, cells were fixed, incubated with anti-human antibodies, and stained with biotinylated anti-mouse or rabbit Ig antibodies, and with FITC and Texas red-avidin with DAPI counterstaining, and observed using an epifluorescence microscope (Olympus). The marker proteins were vascular endothelial growth factor receptor (VEGFR2/KDR) (Lab-Vision), and platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31) (Invitrogen).

2.8. Confocal laser-scanning microscopy

Confocal microscope analysis of 3-day and 7-day cultured EPC was performed as described [9,15]. Briefly, fixed and permeabilized EPC was incubated with specific antibodies against Vimentin (1:1000; Sigma Aldrich) and against SIRT1 (1:500, Abcam) or against CD31 (1:500, FITC; Biolegend) and KDR (1:500, Cell Signaling). Secondary antibodies were Alexafluor 633 (1:1000) or Alexafluor 488 (1:1000). Cells were counterstained with DAPI. Microscopy analyses were performed using Zeiss LSM 510 confocal microscope equipped with a plan-apochromat X 63 (NA 1.4) oil immersion objective [9,15].

2.9. Laboratory analysis

Immediately before PCI and after insulin infusion (24 h) as well as at 7, 30 and 180 days after PCI, glucose levels were measured both in plasma and blood. The evaluations of blood glucose were provided by glucometers "plasma equivalent". All the glucometers used showed satisfactory imprecision and were reliable in reporting plasma-equivalent glucose concentrations. The differences between glucose measured in the lab (plasma glucose) and glucose measured with meter were less than 5%. HbA1c levels were assayed with the high-performance liquid chromatography (HPLC). The determinations were performed by operators who were unaware of the study groups to which the patients were assigned.

2.10. Study end point

The primary end point of the study was the salvage index. The secondary outcome measure was EPC number and capability to differentiate in the early phase of STEMI. The original primary endpoint was in-stent restenosis, but, during analysis, we noted that in patients without restenosis the infarct size was lower in normoglycemic than hyperglycemic patients. Therefore we decided to adopt as co-primary endpoint the salvage index in patients without restenosis.

3. Statistical analysis

All calculations were performed using the computer program SigmaStat 3.5. Continuous variables were expressed as the mean ± SD and were analyzed for significant differences using the two-tailed Student *t* test, when comparing IGC and CGC groups. Categorical variables were expressed as percentages and were analyzed for significant differences using Pearson's chi-square test, two-tailed Fisher exact test as appropriate. To determine the independent predictors of myocardial salvage, all variables with a p value <0.2 (included the amount of insulin infused) were included in a multiple stepwise regression analysis. The sample size had a power of 40% estimated according to a global effect size of 25% with type I error of 0.05. Therefore a sample size of 50 patients per group was necessary.

4. Results

4.1. Baseline characteristics of the patients on admission at emergency wards

A total of 402 patients with suspected STEMI were transferred to or admitted directly to PCI center. Flow diagram of a multicentre trial was reported in Fig. 1. Briefly, hundred-two patients were excluded because primary PCI was not performed, 79 patients were excluded due to a delay in the treatment greater than 24 h, 12 patients were unwilling to provide clinical information, and 8 subjects had their plasma samples unavailable for biochemical analysis. During the first 30 days after PCI, 7 patients died. Therefore, 194 patients completed the study: 88 normoglycemic patients (glucose $<$ 140 mg/dl) (control group) and 106 hyperglycemic patients (glucose $\geq$ 140 mg/dl). Hyperglycemic patients were randomized to intensive glycemic control (IGC) for almost 24 h after PCI (n = 54; glucose 80–140 mg/dl) or conventional glycemic control (CGC, n = 52; glucose 180–200 mg/dl). On admission, despite the similar electrocardiogram alteration on admission in the 3 groups of patients, troponin I levels were significantly higher in patients with hyperglycemia than in normoglycemic patients (Table 1) ($P < 0.005$). There was no correlation between the time from the

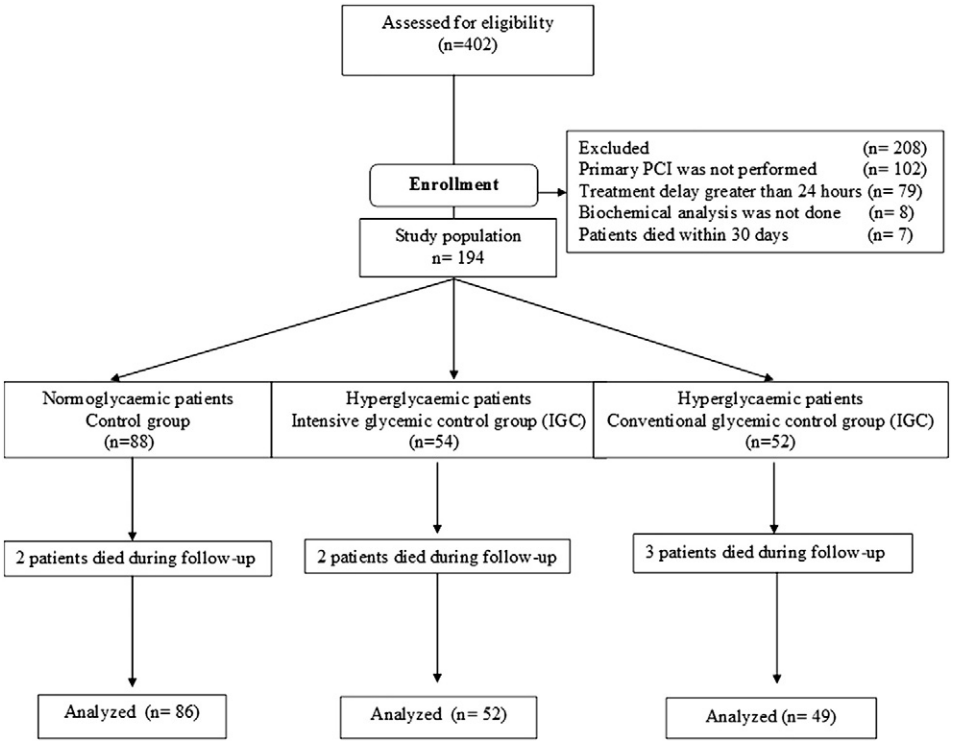

Fig. 1. Flow diagram of the progress through the phases of a parallel randomized trial of groups.

Table 1
Clinical characteristics, angiographic and procedural data.

	Baseline			6 months		
	Control group	IGC group	CGC group	Control group	IGC group	CGC group
N	88	54	52	86	52	49
Mean age (years)	66 ± 11	66 ± 13	65 ± 12	66 ± 13	65 ± 9	66 ± 14
Sex (M/F)	48/40	29/25	27/25	46/40	28/24	25/24
BMI (kg/m^2)	28 ± 2.9	27 ± 3.1	28 ± 2.0	28 ± 2.6	27 ± 3.7	28 ± 2.4
Waist-to-hip ratio	0.73 ± 00.8	0.72 ± 0.09	0.71 ± 0.08	0.72 ± 00.6	0.72 ± 0.07	0.71 ± 0.09
Systolic blood pressure (mmHg)	130 ± 14	129 ± 13	128 ± 14	130 ± 11	129 ± 12	128 ± 14
Diastolic blood pressure (mmHg)	79 ± 10	77 ± 6	78 ± 8	79 ± 12	77 ± 8	78 ± 9
Heart rate (bpm)	85 ± 15	84 ± 18	85 ± 16	84 ± 12	84 ± 19	85 ± 15
Killip class, n (%)						
I	79 (90)	48 (89)	47 (90)	/	/	/
II	6 (7)	4 (8)	4 (7)	/	/	/
III	3 (3)	2 (3)	1 (3)	/	/	/
Risk factors						
Diabetes, n (%)	9 (10)	20 (37)*	20 (38)*	8 (9)	51 (98)*	49 (100)*
Hypertension, n (%)	51 (58)	31 (57)	29 (55)	50 (57)	31 (60)	27 (55)
Hyperlipidemia, n (%)	22 (25)	13 (25)	14 (27)	24 (25)	12 (23)	12 (25)
Obesity, n (%)	30 (34)	20 (37)	19 (37)	30 (34)	20 (38)	19 (39)
Cigarette smoking, n (%)	20 (23)	11 (21)	10 (19)	2 (2)	2 (4)	1 (2)
Active treatments						
β-Blockers, n (%)	11 (12)	8 (14)	7 (14)	63 (72)†	38 (74)†	36 (74)†
ACE inhibitors, n (%)	19 (22)	12 (23)	10 (20)	37 (42)†	22 (43)†	22 (45)†
Angiotensin receptor blockers, n (%)	27 (31)	18 (33)	18 (34)	28 (32)	18 (34)	17 (35)
Statins, n (%)	40 (46)	26 (48)	25 (49)	84 (96)†	51 (98)†	48 (99)†
Thiazide diuretic, n (%)	8 (9)	5 (9)	5(10)	8 (9)	5 (10)	5 (10)
Insulin, n (%)	4 (5)	10 (19)*	9 (18)*	2 (2)	15 (29)*†	17 (35)*†
Oral antidiabetic, n (%)	5 (6)	10 (19)*	11 (21)*	7 (8)	45 (86)*†	44 (89)*†
Aspirin, n (%)	66 (75)	51 (94)*	49 (95)*	83 (95)†	49 (96)	48 (98)
Clopidogrel, n (%)	83 (94)	51 (94)	50 (96)	83 (95)	50 (97)	47 (97)
Fibrinolitic therapy before PCI, n (%)	23 (26)	14 (26)	15 (28)	/	/	/
Laboratory analyses						
Fasting blood glucose, mg/dl	102 ± 14	233 ± 32*	230 ± 34*	97 ± 9	143 ± 20*†	139 ± 22*†
Post-prandial blood glucose, mg/dl	/	/	/	131 ± 12	177 ± 21*	175 ± 26*
HbA1c, %	5.1 ± 0.1	8.7 ± 3.1	8.8 ± 3.0	5.2 ± 0.1	7.3 ± 1.2	7.1 ± 1.6
HbA1c (mmol/mol)	32 ± 2	72 ± 10*	73 ± 9*	33 ± 3	56 ± 10*†	54 ± 11*†
LDL-cholesterol (mg/dl)	112 ± 20	117 ± 13	118 ± 14	102 ± 11	113 ± 12	110 ± 11
HDL-cholesterol (mg/dl)	42 ± 12	39 ± 16	40 ± 14	43 ± 10	41 ± 11	41 ± 16
Triglycerides (mg/dl)	159 ± 44	174 ± 84	178 ± 90	161 ± 46	166 ± 32	168 ± 27
Creatinine (mg/dl)	1.0 ± 0.6	1.1 ± 0.6	1.0 ± 0.8	1.0 ± 0.5	1.1 ± 0.7	1.0 ± 0.7
Troponin (μg/L)	8.9 ± 2.4	13.4 ± 3.8*	13.1 ± 3.6*	/	/	/
Procedural data						
Symptom onset to enrollment, h	1.8 ± 0.4	1.8 ± 0.3	1.9 ± 0.5	/	/	/
Symptom onset to PCI, h	2.6 ± 0.9	2.4 ± 0.7	2.4 ± 0.9	/	/	/
PCI to starting insulin infusion, min	/	31 ± 8	30 ± 9	/	/	/
Stent usage, no. (%)						
Drug-eluting	44 (50)	27 (50)	27 (51)	/	/	/
Bare metal	44 (50)	27 (50)	25 (49)	/	/	/
Number of diseased vessels, n (%)						
1-VD	53 (60)	33 (61)	32 (62)	/	/	/
2-VD	26 (30)	17 (32)	17 (33)	/	/	/
3-VD	9 (10)	4 (7)	3 (5)	/	/	/
Lesion location, n (%)						
LAD	43 (49)	26 (48)	25 (49)	/	/	/
LCx	32 (36)	20 (37)	18 (35)	/	/	/
RCA	19 (22)	11 (21)	12 (23)	/	/	/
Type of lesion, %						
A	11 (12)	6 (12)	7 (13)	/	/	/
B	28 (32)	18 (33)	17 (32)	/	/	/
C	49 (56)	30 (55)	29 (56)	/	/	/
PCI procedure						
Nominal size of largest balloon, mm	2.87 ± 0.43	2.89 ± 0.42	2.87 ± 0.43	/	/	/
Balloon to artery ratio	1.15 ± 0.20	1.15 ± 0.18	1.14 ± 0.21	/	/	/
Total number of inflations	3.8 ± 3.1	3.7 ± 3.4	3.7 ± 3.3	/	/	/
Total duration of inflation, s	312 ± 151	310 ± 145	308 ± 144	/	/	/
Maximum inflation pressure, atm	11.6 ± 2.4	11.5 ± 2.3	11.4 ± 2.9	/	/	/
Quantitative angiographic data						
Before angioplasty						
Lesion length, mm	13.9 ± 4.9	14.9 ± 6.7	14.7 ± 7.1	/	/	/
Reference diameter, mm	3.0 ± 0.7	2.8 ± 0.6	2.8 ± 0.4	/	/	/
MLD, mm	0.6 ± 0.4	0.6 ± 0.6	0.6 ± 0.5	/	/	/
Percentage stenosis, %	77 ± 12	79 ± 16	78 ± 15	/	/	/

(continued on next page)

Table 1 (*continued*)

	Baseline			6 months		
	Control group	IGC group	CGC group	Control group	IGC group	CGC group
Quantitative angiographic data						
After angioplasty						
Reference diameter, mm	2.6 ± 0.6	2.7 ± 0.9	2.7 ± 0.7	/	/	/
MLD, mm	2.7 ± 0.9	2.6 ± 0.7	2.7 ± 0.6	/	/	/
Percentage stenosis, %	21 ± 14	23 ± 16	24 ± 12	/	/	/
Hospital stay (days)	6 ± 2	7 ± 3	7 ± 2	/	/	/
Scintigraphic evaluation						
Initial defect size, % LV (25th–75th percentiles)	20 (16–26)	29 (26–33)*	30 (26–34)*	/	/	/
Final infarct size, % LV (25th–75th percentiles)	/	/	/	10 (7.5–11)	14 (14–16)*	22 (19–24)*‡
Myocardial salvage, % LV (25th–75th percentiles)	/	/	/	11 (7–17)	15 (10–19)*	7 (4–12)*‡
Salvage index, (25th–75th percentiles)	/	/	/	0.6 (0.4–0.7)	0.5 (0.4–0.6)	0.2 (0.1–0.4)*‡

Data are means ± SD, n (%) or median (percentiles). 1-VD indicates single-vessel disease; 2-VD, two-vessel disease; 3-VD, three-vessel disease; LAD, left anterior descending; LCx, left circumflex artery; RCA, right coronary artery; and PCI: percutaneous coronary intervention; MLD, minimal luminar diameter.

* $P < 0.05$ vs control group.

† $P < 0.05$ vs baseline.

‡ $P < 0.05$ vs IGC group.

onset of symptoms and the admission troponin I levels ($R2 = 0.007$, $P = 1.00$). Pretreatment with fibrinolysis was used in 23 patients (26%) of control group, 14 patients (26%) of IGC group and 15 patients (28%) of CGC group. The admission plasma glucose level was similar among IGC and CGC groups (Table 1). There were no differences in the mean age, BMI, sex distribution, smoking habits, HbA1c, plasma cholesterol, and triglyceride levels among the 3 groups. The use of diuretic drugs, angiotensin-converting enzyme inhibitor drugs, beta-blocker drugs, and calcium channel blocker therapy was similar among the 3 groups (Table 1). A total of 38% of patients in the CGC group and 37% of patients in the IGC group had been previously treated for diabetes, while the remaining patients were newly diagnosed with diabetes. The number (Fig. 2) and differentiation of EPCs (Fig. 3) as well as SIRT1 levels (Fig. 4) were significantly higher in patients with normoglycemia than in those with in hyperglycemia and healthy subjects. Moreover, there were no significant differences among IGC and CGC groups at baseline. Angiographic data are summarized in Table 1. The treated lesion types were similar in the groups (Table 1). The stent type was similar in the groups (Table 1). Quantitative angiographic analysis is summarized in Table 1. A mean of 2.3 matched angiographic projections per lesion was made, than satisfactory quantitative analysis was performed at the central angiographic laboratory before and after PCI (Table 1). The reference diameter of the target vessel was similar in the groups. The mean minimal luminal diameter, and the mean length of the lesion at baseline were similar in the groups. No significant difference was observed in stent length and stent diameter between the groups (Table 1). The lesion location, AHA/ACC lesion classification, angiographic measurements, and frequency of multi-lesion PCI were similar between patients with and without diabetes (Table 1). Compared to the hyperglycemic patients, the initial perfusion defect was lower in normoglycemic group (Table 1). Correlation between the initial perfusion defect and glucose levels was found ($R = 0.69$; $P < 0.001$).

4.2. Effects of glucose-lowering treatments on EPC number and differentiation, after insulin infusion 1, 7 and 30 and from the PCI in hyperglycemic patients

There were no difference in the mean time between PCI and starting insulin infusion among the groups. In both groups, the mean time required to achieve plasma glucose target was 8.4 ± 2.1 h in normoglycemic patients, 8.6 ± 2.2 h in IGC and 8.8 ± 2.5 h in CGC group. In hyperglycemic patients, the insulin infusion duration was 33.9 ± 2.5 h

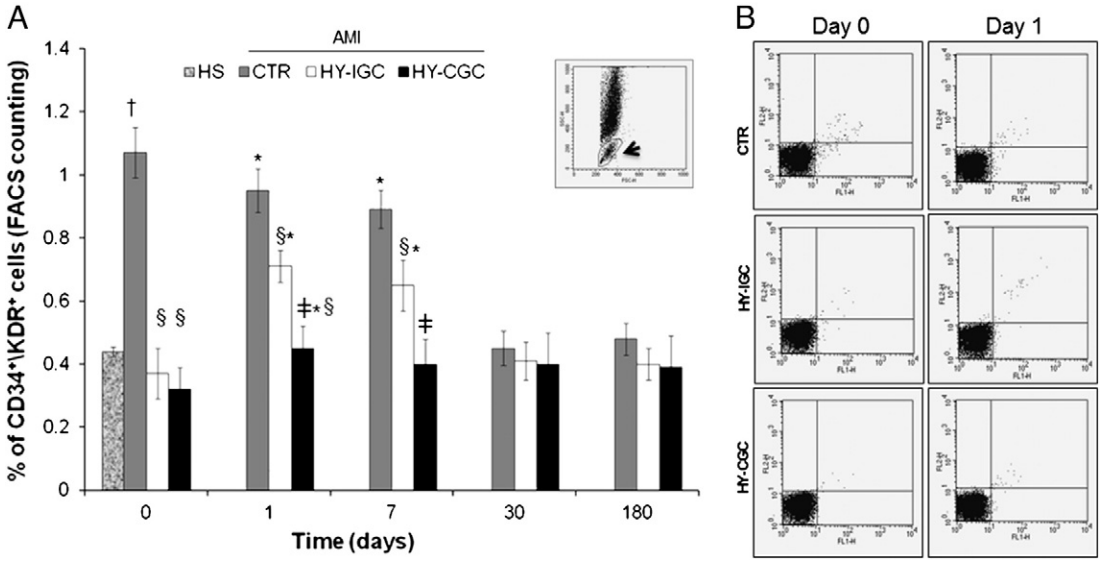

Fig. 2. FACS computed counting of EPCs. (A) Characterization and counting of circulating EPCs by FACS analysis of the co-expression of CD34/KDR (VEGFR2). (B) Representative FACS plots and gating profile set on the basis of the isotype control sample as used for the quantification of and $CD34^+KDR^+$ cells. FL1 = CD34-FITC; FL2 = KDR-PE. Data are mean ± SD, with *P < 0.01 vs baseline, §p < 0.05 vs CRT, ‡P < 0.05 vs IGC, †P < 0.05 vs HS. HS = healthy subjects; CTR = control; HY-IGC = hyperglycaemic-intensive glycemic control; HY-CGC = conventional glycemic control.

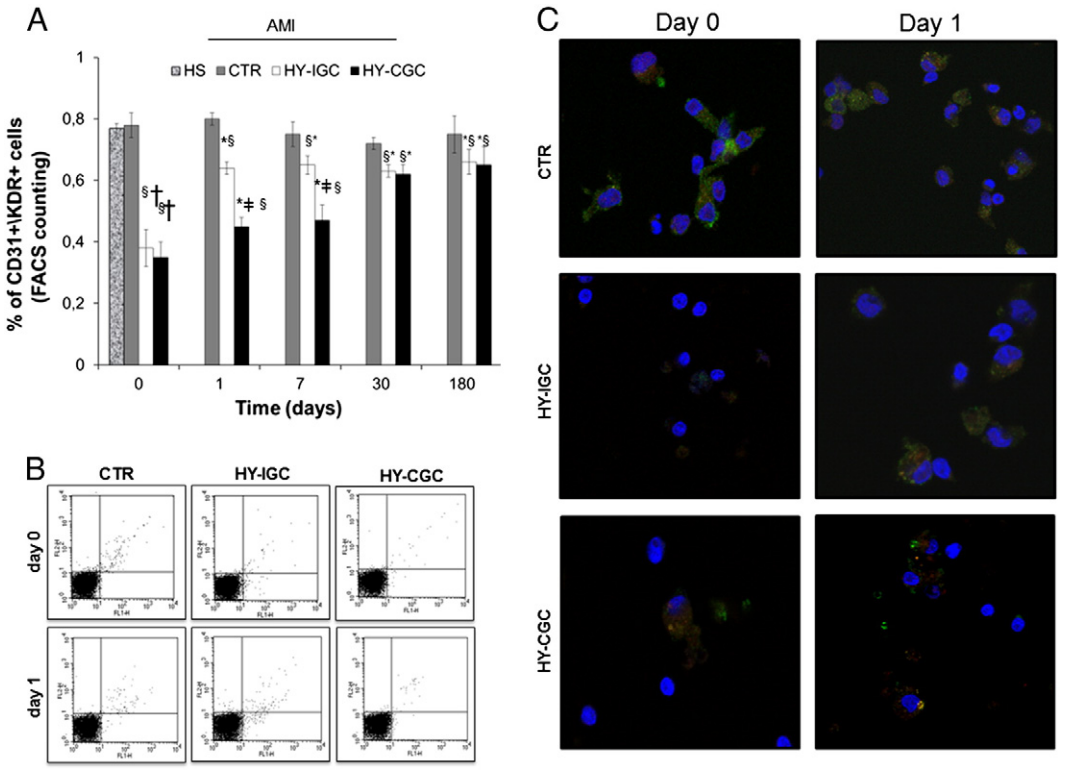

Fig. 3. FACS computed counting and confocal laser-scanning microscopy evaluation of EPCs differentiation. (A) FACS analysis of 7-day cultured EPCs ($CD31^+/KDR^+$) from AMI-patients (CTR = control; HY-IGC = hyperglycemic-intensive glycemic control; HY-CGC = conventional glycemic control) and healthy subjects (HS). (B) Representative FACS plots and gating profile set on the basis of the isotype control sample as used for the quantification of and $CD31^+KDR^+$ cells. (C) Representative confocal laser-scanning images of cells co-expressing $CD31^+$ (green) and KDR^+ (red). Nuclei were counterstained with DAPI (blue). Data are mean ± SD, with *P < 0.01 vs baseline, §P < 0.05 vs CRT, ‡P < 0.05 vs IGC, †P < 0.05 vs HS.

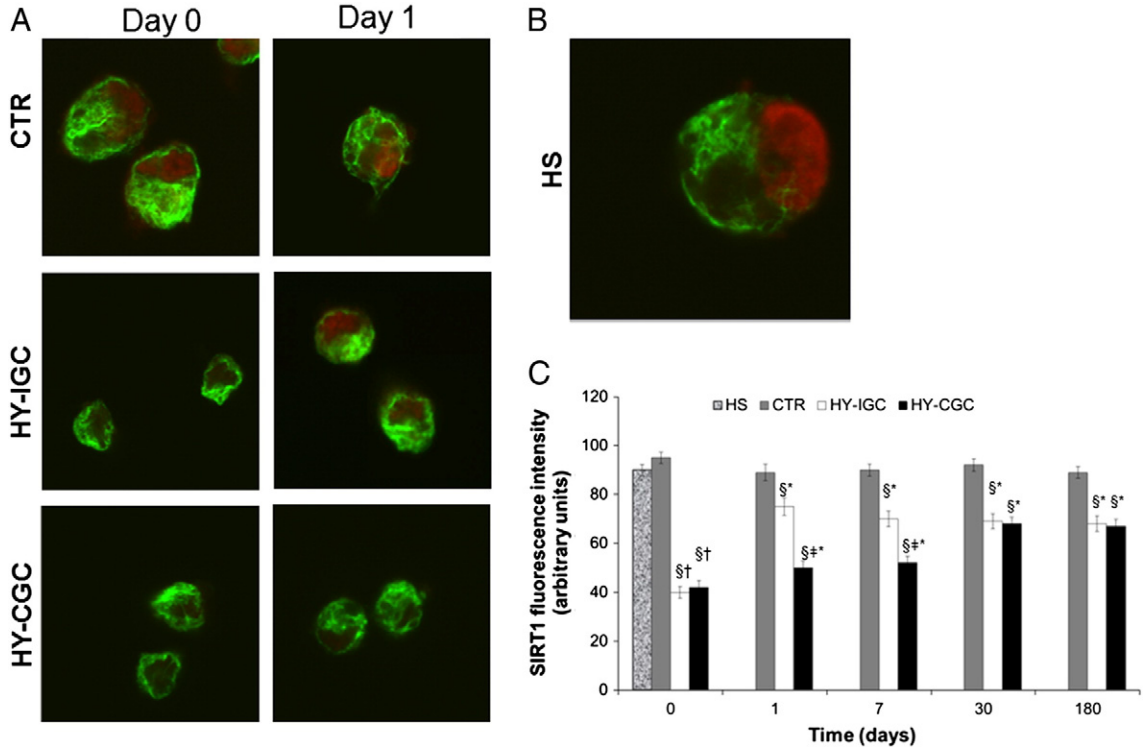

Fig. 4. Evaluation of SIRT1 expression in EPCs. Representative confocal images of EPCs from (A) AMI-patients (CTR = control; HY-IGC = hyperglycaemic-intensive glycemic control; HY-CGC = conventional glycemic control) and (B) Healthy subjects (HS). EPCs were incubated with specific antibodies against vimentin (green) and SIRT1 (red). Secondary antibodies were Alexa Fluor 488 and Alexa Fluor 633. (C) Immunofluorescence analysis of SIRT1 (red) and vimentin (green) protein levels. The fluorescence intensity was calculated with ImageJ software analysis and expressed as arbitrary fluorescence units (AFU). Alexa 488 and Alexa 633 fluorescence emissions were acquired in multi-track mode using BP 505–530 and LP650 filters respectively. The mean (n = 6) of the unspecific fluorescence of the secondary antibodies was calculated with ImageJ software and subtracted. Data are mean ± SD, with *P < 0.01 vs baseline, §P < 0.05 vs CRT, ‡P < 0.05 vs IGC, †P < 0.05 vs HS.

in IGC group and 33.6 ± 2.2 h in CGC group. The amount of insulin infused was 111 ± 29 IU in the IGC patients and 48 ± 7 IU in the CGC patients. After the insulin infusion period, plasma glucose reduction was greater in the IGC group than in CGC group (89 ± 29 mg/dl vs 27 ± 22 mg/dl; $P < 0.001$). Therefore, the mean plasma glucose level during the peri-procedural period was greater in the CGC group than in IGC group (CGC, 201 ± 16 mg/dl; IGC, 144 ± 36 mg/dl; $P < 0.001$). Moreover, the glycemic goal was maintained for 24 h in both groups (ICG, 129 ± 10 mg/dl; CGC, 197 ± 12; $P < 0.01$). Blood glucose <70 mg/dl with and without symptoms occurred more frequently during the insulin infusion in IGC group (33%) than in CGC group (9%). After insulin infusion, multidose insulin therapy was used in all ICG patients (46 ± 13 IU/day) and in the 21% (n = 25) of CGC patients (44 ± 18 IU/day) during the whole in-hospital period (ICG, 14 ± 6 days; CGC, 14 ± 7 days). During the hospital stay, blood glucose <70 mg/dl with and without symptoms occurred more frequently in IGC group (10%) than in CGC group (3%). Fig. 1 shows the time course of, ECP number (Fig. 2), differentiation capability (Fig. 3), and EPC SIRT1 protein level (Fig. 4), were still greater in normoglycemic patients than in healthy subjects and in IGC and CGC patients. After the insulin infusion period (24 h) and on day 7 following STEMI, the number and differentiation of EPCs and SIRT1 levels were higher in the IGC patients compared to CGC patients (Figs. 2, 3, and 4). Moreover, EPC number and differentiation were significantly associated with mean plasma glucose level during the insulin infusion period, and regression model showed a positive nonlinear effect ($R = 0.31$, $P < 0.001$; $R = 0.29$, $P < 0.001$). At hospital discharge, both fasting and post-prandial blood glucose levels were higher in CGC group than in ICG group (IGC: fasting, 116 ± 20 mg/dl, post-prandial 168 ± 16 mg/dl; CGC: 147 ± 24 mg/dl, post-prandial 199 ± 18 mg/dl; $P < 0.05$ for all). At hospital discharge, all patients with established type 2 diabetes (IGC, 20 patients; CGC, 20 patients) and newly diagnosed type 2 diabetes (IGC, 24 patients; CGC, 22 patients) were managed and followed as outpatients for 6 month after PCI (Table 1). After 30 days from the PCI, both fasting and post-prandial plasma glucose levels were similar in the two groups (IGC: fasting, 133 ± 28 mg/dl, post-prandial 177 ± 22 mg/dl; CGC: 131 ± 26 mg/dl, post-prandial 175 ± 31 mg/dl; P = NS for both). After 30 days from intervention treatment period, there were no differences in the hypoglycemic events among the groups (data not shown). After 30 days of intervention treatment period, the number of EPCs was similar in normoglycemic and hyperglycemic group without differences among IGC and CGC groups (Fig. 2). The differentiation of EPC (Fig. 3) and SIRT1 protein level (Fig. 4) were higher in normoglycemic than hyperglycemic groups. However, there were no differences among IGC and CGC group.

4.3. Effects of glucose-lowering treatments on the myocardial salvage, after 180 days from the PCI

There was no difference in HbA1c (P = NS) at 6 months among the patients from both hyperglycemic groups. There was no difference in hypoglycemic and medical out-patient treatment therapy during the follow-up among the groups (IGC, 14%; CGC, 15%). After 180 days from the PCI, both fasting and post-prandial plasma glucose levels were similar in the two groups (IGC: fasting, 143 ± 20 mg/dl, post-prandial 177 ± 21 mg/dl; CGC: 139 ± 22 mg/dl, post-prandial 175 ± 26 mg/dl; P = NS for both). After 180 days of intervention treatment period, the number of EPCs was similar in normoglycemic and hyperglycemic group without differences among IGC and CGC groups (Fig. 2). The differentiation of EPC (Fig. 3) and SIRT1 protein expression levels (Fig. 4) was higher in normoglycemic than hyperglycemic groups, however, there were no differences among IGC and CGC group. Myocardial salvage index was compared between normoglycemic and hyperglycemic patients (Table 1, Fig. 5). The myocardial salvage index was similar in normoglycemic and in IGC patients. In the hyperglycemic group, the salvage index was significantly greater among IGC patients as compared with the CGC patients (Table 1, Fig. 4). Moreover, EPC number and differentiation changes during the first 24 h after STEMI were significantly associated with myocardial salvage index, and regression model showed a positive nonlinear effect ($R = 0.41$, $P < 0.001$; $R = 0.49$, $P < 0.001$). In addition, using the data pertaining to the entire study population, we built a multiple linear regression model with salvage index as a dependent variable and age, sex, diabetes, HbA1c, mean plasma glucose level during the insulin infusion period and the other cardiovascular risk factors, infarct localization, proportion of ST-elevation infarction, time-to admission, door-to-reperfusion treatment time. After adjustment in the multivariate model mean plasma glucose level during the insulin infusion period remained an independent predictor Of the salvage index ($P < 0.02$). By 6 months, mortality was 2.2% (n = 2) in the control group, 3.7% (n = 2) in the IGC group and 5.7% (n = 3) in the CGC group.

5. Discussion

The main message of the present study, on different goal for glucose control in patients with hyperglycemia submitted to PCI for STEMI, is that peri-procedural tight glycemic control significantly increased the area of myocardial salvage accompanied with a reduction of the ischemic area and greater recovery of LV function at 6 months after stenting. Tight glycemic control, for at least 24 h, is associated with a doubled increase in myocardial salvage in the IGC patients compared to that of the CGC group, despite both the intensive and conventionally treated groups returned to their usual glucose lowering management at the end of the active phase of the study. These data, consistent with those of previous studies, support the proposition that stress hyperglycemia was associated with impaired myocardial salvage in patients on admission for AMI [2]. Furthermore, we found that the tight control of peri-procedural plasma glucose levels were associated with improved myocardial salvage independently from HbA1c levels. These observations suggest that the acute metabolic milieu at the time of the PCI is more significant than chronic glycemic control in setting the stage for myocardial salvage. A support for the potential benefit of early optimal glycemic control during PCI to improve myocardial salvage at 6 months may also be derived from clinical studies suggesting that the injurious effects of exposure to high glucose levels persist for years after better treatment, a phenomenon typically referred to a hyperglycemic memory [16]. Of particular interest were the findings from the Diabetes Control and Complications Trial and its follow-up observational study, the Epidemiology of Diabetes Intervention and Complications trial where the term “hyperglycemic memory” was introduced [17,18]. This term was used to explain a phenomenon where the long-term vascular benefits of a previous period of good glycemic control persisted despite a return to usual, often worse metabolic control. In this context, experimental evidence indicates that short-term memory of hyperglycemic conditions is associated with long-term changes in chromatin modifications [19]. Indeed, short-term exposure to high-glucose concentration showed a sustained decrease in the number and the functional properties of EPCs in diabetic patients [9]. In the process of myocardial regeneration, bone marrow-derived and peripheral tissue-derived progenitor cells appear to be able to regenerate a myocardium by enhancing the neovascularization [20,21]. These pro-angiogenic mechanisms may allow EPCs to positively contribute to the regeneration of an ischemic myocardium after reperfusion therapy. Moreover, recent studies have found that the magnitude of circulating EPCs appearing as $CD34^+/KDR^+$ after MI may predict the clinical outcome and prognosis [21,22]. Regarding EPC function, we first measured the effect of thigh glycemic control on the abundance and differentiation capability of EPCs of hyperglycemic patients with AMI and compared them with clinical outcome parameters. In accordance with the literature [5–7], we have shown that there is a considerable increase of EPC number early after AMI in normoglycemic patients. However, we demonstrate that the increase of EPC levels is affected in hyperglycemic patients early after

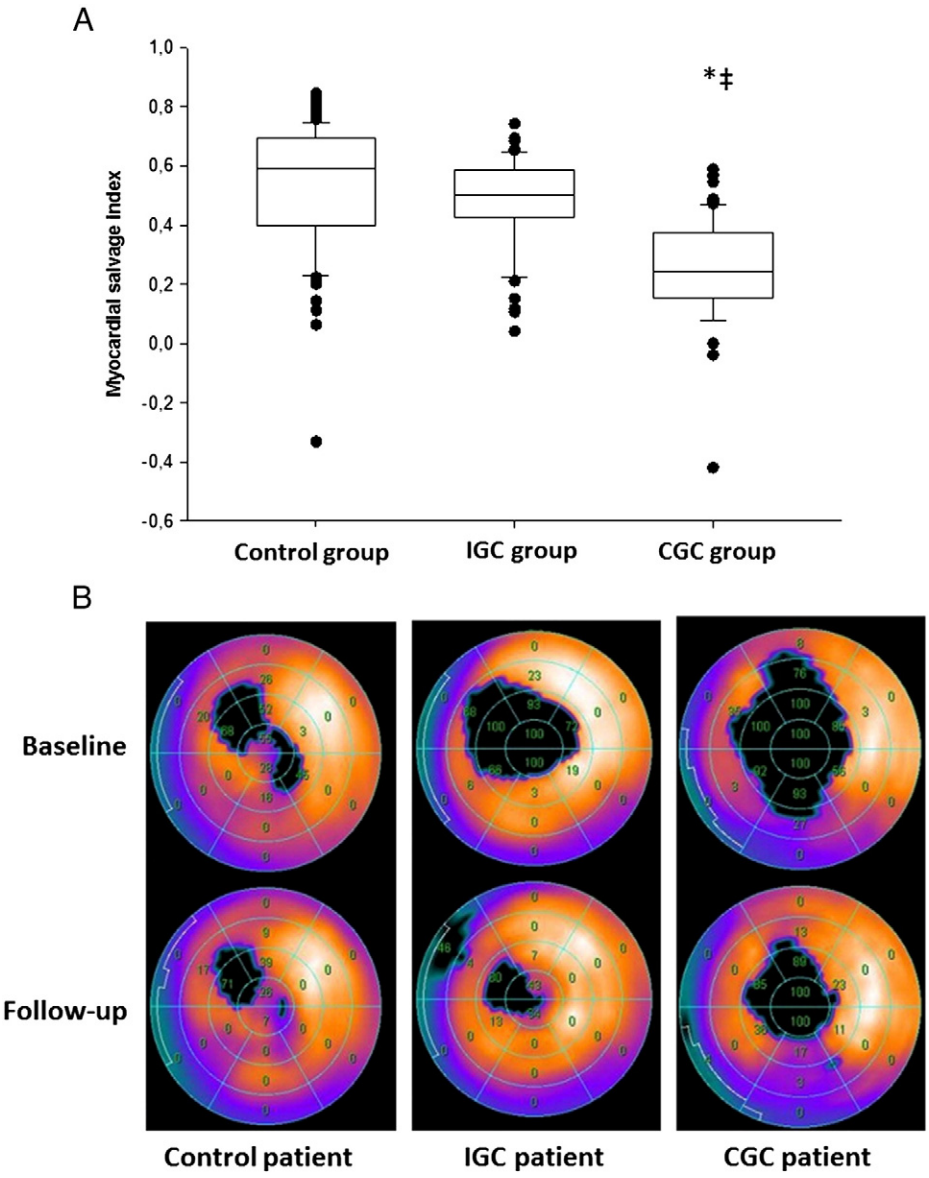

Fig. 5. Myocardial salvage between the groups. (A) Salvage index in the control group, intensive glycemic control group (IGC) and conventional glycemic control group (CGC). In all box plots, the top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile. The whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers (values that are between 1.5 and 3 times the interquartile range) and extreme values (values that are more than 3 times the interquartile range) are represented by circles beyond the whiskers. *$P < 0.05$ vs control group. ‡$P < 0.05$ vs IGC group. (B), Representative cases of technetium Tc-99m sestamibi scintigraphy from each group at baseline (5 to 7 days after STEMI) and at follow-up (180 days after STEMI).

STEMI, compared to normoglycemic patients. Regarding the mobilization and the function of EPC hyperglycemic patients, the increased level and differentiation of EPCs after insulin infusion were greater in IGC than in CGC patients. These differences among IGC and CGC patients continued until seven days after STEMI. Since a rapid and considerable increase of circulating EPCs, which occurs during acute ischemia, correlates with healing and functional recovery of injured areas [6], it has been hypothesized that pharmacologic interventions leading to glycemic control may influence the recovery of myocardial function and other yet-unrecognized mechanisms through the amelioration of the EPC number and functionality. These effects may result in an increased myocardial salvage. As for mechanisms behind this association, we evidenced that during STEMI a downregulation of SIRT1 levels is observed in hyperglycemic patients compared to normoglycemic patients. Several cellular processes including insulin secretion, cell cycle, and apoptosis are imperatively regulated by a family of mediators called sirtuins. Among sirtuins, first known mammalian sirtuin, SIRT1, is a positive regulator of EPC mobilization and differentiation [23,24,15]. SIRT1 actions may affect cellular pathways involved in aging and metabolic diseases. In particular, evidence suggests an important role for SIRT1in shear stress-induced EPC differentiation [25]. In this framework, our data suggest that hyperglycemia-induced downregulation of SIRT1 may play a pivotal role in the reduced number and function of EPC in hyperglycemic patients during STEMI. Accordingly, previous study evidences that SIRT1 protein level and activity are decreased in type 2 diabetic patients with poor glycemic control with respect to patients with good glycemic control [9]. Since SIRT1 has been shown to play a pivotal role in the differentiation of EPCs, detection of low levels of SIRT1 in CGC patients but not in IGC patients is strongly suggestive of reduced EPC number and function and, thus, of impaired myocardial function linked to a decreased availability of EPC function during STEMI. According to this construct, we evidenced that tight glycemic control (129 ± 10 mg/dl) in the immediate PCI procedure period, for almost 24 h, was associated with the up-regulation of SIRT1. Tight glycemic control was associated with higher levels of both SIRT1 and EPC number in IGC patients compared to CGC patients. In particular, we proved

evidence of the increase of EPC number and differentiation capacity, as well as SIRT1 protein level, only after insulin infusion and not during follow-up. In this context, the increased EPC number and functionality, i.e., differentiation capability and SIRT1 protein level, evoked by tight glycemic control in the peri-procedural period may play a critical role in contributing to an increased myocardial salvage at 6 months. But, above all, it is very intriguing that our study parameters of EPC number and differentiation assessed after insulin infusion, similarly to mean glycemic values, were correlated with myocardial salvage index at 180 days after PCI. Therefore, these findings strongly support the hypothesis that an increase of EPC level and capability of differentiation could take part to a favorable effect of peri-procedural tight glycemic control during early percutaneous coronary intervention on myocardial salvage in patients with acute ST-elevation myocardial infarction.

6. Study limitation

The limited number of patients as well as the short period of observation are the limitations of our study. Therefore, further studies involving a larger number of patients will be needed to confirm our data.

Source of founding

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Contribution statement

M.L.B. and R.M. wrote the manuscript and researched data.
G.P., M.R.R., and M.B. reviewed/edited the manuscript.
N.D.O., P.P., A.S. M.S., C.M., N.A., and N.E. contributed to the discussion and reviewed/edited the manuscript. C. S., N.A., N.E., S.D.G., P.F.R., and L.M. researched data and contributed to discussion. All authors provided the final approval of the version to be published.

References

[1] Capes SE, Hunt D, Malmberg K, Gerstein HC. Stress hyperglycaemia and increased risk of death after myocardial infarction in patients with and without diabetes: a systematic overview. Lancet 2000;355:773–8.
[2] Teraguchi I, Imanishi T, Ozaki Y, et al. Impact of stress hyperglycemia on myocardial salvage following successfully recanalized primary acute myocardial infarction. Circ J 2012;76:2690–6.
[3] Malmberg K, Ryden L, Efendic S, et al. A randomised trial of insulin-glucose infusion followed by subcutaneous insulin treatment in diabetic patients with acute myocardial infarction: effects on mortality at 1 year. J Am Coll Cardiol 1995;26:57–65.
[4] Deedwania P, Kosiborod M, Barrett E, et al. Hyperglycemia and acute coronary syndrome: a scientific statement from the American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity, and Metabolism. Circulation 2008;117:1610–9.
[5] Liu W, Peng Y, Wu B, et al. A Meta-Analysis of the Impact of EPC Capture Stent on the Clinical Outcomes in Patients with Coronary Artery Disease. J Interv Cardiol 2013;26:228–38.
[6] Leone AM, Rutella S, Bonanno G, et al. Mobilization of bone marrow-derived stem cells after myocardial infarction and left ventricular function. Eur Heart J 2005;26:1196–204.
[7] Numaguchi Y, Sone T, Okumura K, et al. The impact of the capability of circulating progenitor cell to differentiate on myocardial salvage in patients with primary acute myocardial infarction. Circulation 2006;114(1 Suppl.):I114–9.
[8] Ling L, Shen Y, Wang K, et al. Worse clinical outcomes in acute myocardial infarction patients with type 2 diabetes mellitus: relevance to impaired endothelial progenitor cells mobilization. PLoS One 2012;7:e50739.
[9] Balestrieri ML, Servillo L, Esposito A, et al. Poor glycaemic control in type 2 diabetes patients reduces endothelial progenitor cell number by influencing SIRT1 signalling via platelet-activating factor receptor activation. Diabetologia 2013;56:162–72.
[10] Fadini GP, Miorin M, Facco M, et al. Circulating endothelial progenitor cells are reduced in peripheral vascular complications of type 2 diabetes mellitus. J Am Coll Cardiol 2005;45:1449–57.
[11] Mortuza R, Chen S, Feng B, Sen S, Chakrabarti S. High glucose induced alteration of SIRTs in endothelial cells causes rapid aging in a p300 and FOXO regulated pathway. PLoS One 2013;8:e54514.
[12] Mylonas I, Beanlands RS. Radionuclide imaging of viable myocardium: is it underutilized? Curr Cardiovasc Imaging Rep 2011;4:251–61.
[13] Kumar A, Cannon CP. Acute coronary syndromes: diagnosis and management. Mayo Clin Proc 2009;84:917–38.
[14] Marfella R, Sasso FC, Siniscalchi M, et al. Peri-procedural tight glycemic control during early percutaneous coronary intervention is associated with a lower rate of in-stent restenosis in patients with acute ST-elevation myocardial infarction. J Clin Endocrinol Metab 2012;97:2862–71.
[15] Balestrieri ML, Rienzo M, Felice F, et al. High glucose downregulates endothelial progenitor cell number via SIRT1. Biochim Biophys Acta 2008;1784:936–45.
[16] Ceriello A, Ihnat MA, Thorpe JE. Clinical review 2: the “metabolic memory”: is more than just tight glucose control necessary to prevent diabetic complications? J Clin Endocrinol Metab 2009;94:410–5.
[17] The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. N Engl J Med 2000;342:381–9.
[18] Writing Team for the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Research Group. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. JAMA 2003;290:2159–67.
[19] El-Osta A, Brasacchio D, Yao D, et al. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med 2008;205:2409–17.
[20] Shintani S, Murohara T, Ikeda H, et al. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 2001;103:2776–9.
[21] Wojakowski W, Tendera M, Michalowska A, et al. Mobilization of CD34/CXCR4^{+}, CD34/CD117^{+}, c-met+ stem cells, and mononuclear cells expressing early cardiac, muscle, and endothelial markers into peripheral blood in patients with acute myocardial infarction. Circulation 2004;110:3213–20.
[22] Massa M, Rosti V, Ferrario M, et al. Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. Blood 2005;105:199–206.
[23] Brunet A, Sweeney LB, Sturgill JF, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004;303:2011–5.
[24] Fiorito C, Rienzo M, Crimi E, et al. Antioxidants increase number of progenitor endothelial cells through multiple gene expression pathways. Free Radic Res 2008;42:754–62.
[25] Cheng BB, Yan ZQ, Yao QP, et al. Association of SIRT1 expression with shear stress induced endothelial progenitor cell differentiation. J Cell Biochem 2012;113:3663–71.