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# Exenatide reduces reperfusion injury in patients with ST-segment elevation myocardial infarction

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**Aims** Exenatide, a glucagon-like-peptide-1 analogue, increases myocardial salvage in experimental settings with coronary occlusion and subsequent reperfusion. We evaluated the cardioprotective effect of exenatide at the time of reperfusion in patients with ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI).

**Methods and results** A total of 172 patients with STEMI and Thrombolysis in Myocardial Infarction flow 0/1 were randomly assigned to exenatide or placebo (saline) intravenously. Study treatment was commenced 15 min before intervention and maintained for 6 h after the procedure. The primary endpoint was salvage index calculated from myocardial area at risk (AAR), measured in the acute phase, and final infarct size measured 90 ± 21 days after pPCI by cardiac magnetic resonance (CMR). In 105 patients evaluated with CMR, a significantly larger salvage index was found in the exenatide group than in the placebo group (0.71 ± 0.13 vs. 0.62 ± 0.16; $P = 0.003$). Infarct size in relation to AAR was also smaller in the exenatide group (0.30 ± 0.15 vs. 0.39 ± 0.15; $P = 0.003$). In a regression analysis, there was a significant correlation between the infarct size and the AAR for both treatment groups and an analysis of covariance showed that datapoints in the exenatide group lay significantly lower than for the placebo group ($P = 0.011$). There was a trend towards smaller absolute infarct size in the exenatide group (13 ± 9 vs. 17 ± 14 g; $P = 0.11$). No difference was observed in left ventricular function or 30-day clinical events. No adverse effects of exenatide were observed.

**Conclusion** In patients with STEMI undergoing pPCI, administration of exenatide at the time of reperfusion increases myocardial salvage.

**Keywords** Reperfusion injury • Exenatide • Acute myocardial infarction • Cardiac magnetic • Resonance • Primary percutaneous coronary intervention

## Introduction

ST-segment elevation myocardial infarction (STEMI) is a major cause of mortality and morbidity.[1] The recommended treatment for STEMI is reperfusion therapy with primary percutaneous coronary intervention (pPCI), which reduces mortality and morbidity.[2] However, acute restoration of myocardial blood flow may in itself jeopardize the cardiomyocytes. This phenomenon, known as reperfusion injury, may account for as much as 50% of the final myocardial infarct size,[3] a major determinant of the prognosis in patients with STEMI.[4] In spite of constant improvements in the treatment of patients with acute myocardial infarction, there is still a need to protect the heart during reperfusion.

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Glucagon-like peptide-1 (GLP-1) is an incretin hormone that regulates plasma glucose, and recently GLP-1 analogues have been introduced for the treatment of type-2 diabetes.[5] In addition, receptors for GLP-1 have been found in the heart.[6] In experimental studies, GLP-1 or its analogues protect against reperfusion injury-induced cell death.[7] These cardioprotective analogues include exendin-4, a peptide derived from a lizard venom showing a GLP-1-like potency and efficacy at GLP-1 receptors.[8] Exenatide is a synthetic version of exendin-4 and the active substance in the anti-diabetic drug BYETTA® (Amylin-Lilly, USA). Recently, we found exendin-4 to be cardioprotective during reperfusion in isolated rat hearts,[9] a finding that has been confirmed.[10] However, the protective effect of GLP-1 or its analogues against reperfusion injury has never been investigated in clinical settings.

In a proof-of-concept study, we investigated the cardioprotective effects of intravenous exenatide administered prior to reperfusion and continued after restoration of coronary blood flow in patients with STEMI undergoing pPCI. Cardiac magnetic resonance (CMR) imaging was used to assess the myocardial area at risk (AAR) and the final infarct size.

# Methods

## Trial

This randomized, double-blind, placebo-controlled trial was performed at Copenhagen University Hospital Rigshospitalet, Denmark, and Aarhus University Hospital Skejby, Denmark. All patients were informed orally and in writing, and all gave their written consent before inclusion. The study was performed according to the Helsinki Declaration of Good Clinical Practice, and The Danish National Committee on Biomedical Research Ethics approved the protocol. Exenatide was purchased with institutional grant support; the manufacturer had no impact on the design, execution, or data analysis of the study. The study was registered at www.clinicaltrial.gov; identifier: NCT00835848.

## Study population

Patients were eligible if they were 18 years or older and presented within 12 h from the onset of symptoms and signs of STEMI to the catheterization laboratory. An ECG was obtained either in the ambulance or at the referring hospital. STEMI was defined as significant ST-segment elevation in at least two contiguous leads. The following ST-segment elevation criteria were used: 1 mV ST-segment elevation in the limb lead (II, III and aVF, I, aVL) and $V_4$–$V_6$, and 2 mm ST-segment elevation in $V_1$–$V_3$. The patients were not considered for enrolment if they presented with unconsciousness, cardiogenic shock, hypoglycaemia, diabetic ketoacidosis, previous myocardial infarction, stent thrombosis, known renal insufficiency, or previous coronary artery bypass operation. Furthermore, patients were excluded if they met one of the following angiographic exclusion criteria: any other lesion than the culprit with a diameter stenosis >70% on the coronary angiography and Thrombolysis in Myocardial Infarction (TIMI) flow grade >1 before intervention.

## Angiography, treatment, and primary percutaneous coronary intervention

Patients eligible for pPCI were pre-treated with aspirin (300 mg orally or 500 mg intravenously), clopidogrel (600 mg orally), and heparin (10 000 U intravenously). After randomization, coronary angiography was performed to identify the culprit lesion. Direct stenting, thrombectomy, and choice of stent were left to the discretion of the operator. Predilatation with a small-sized balloon was allowed before stenting. Ischaemic postconditioning was not allowed and balloon angioplasty alone was limited to cases in which a stent could not be deployed or was considered harmful. Glycoprotein IIb/IIIa receptor antagonists were administered when no contraindications were present. All patients were treated with clopidogrel 75 mg daily for 12 months and aspirin 75 mg daily lifelong. Two blinded observers analysed the angiograms for other stenoses than the culprit lesion, collateral flow to the infarct-related artery according to the Rentrop grade classification and TIMI flow grade.

## Experimental treatment protocol

It was assumed that an appropriate plasma concentration of exenatide had to be achieved before reperfusion, which necessitated the treatment to be commenced before angiography. Consequently, randomization to either placebo or exenatide was performed prior to angiography in all patients without pre-angiographic exclusion criteria using a 1:1 computer-generated sequence. Numbered sealed envelopes, containing the study group assignment, were opened after informed consent was obtained. Both the operator and patient were blinded to the allocated treatment before, during, and after the infusion. Patients assigned to exenatide were treated with an intravenous infusion of exenatide BYETTA® (Amylin-Lilly) diluted in saline (25 μg exenatide in 250 ml saline). The infusion was commenced 15 min before intervention with a flow rate of 72 mL/h (0.12 μg/min) to result in exenatide plasma concentration between 0.03 and 0.30 nmol/L at 15 min. The angiography was not delayed by this procedure. After 15 min, the flow rate was reduced to 26 mL/h (0.043 μg/min) and maintained for 6 h in order to maintain exenatide plasma concentration between 0.03 and 0.3 nmol/L. The plasma concentrations of exenatide between 0.03 and 0.30 nmol/L were chosen based a concentration level previously documented to be efficient in our own experimental work.[9] The time of reperfusion was in pilot patients found to be no sooner than 15 min after randomization, and we thus wanted the steady-state plasma concentration to be achieved by this time. The infusion parameters were based on an exenatide distribution volume of 64 mL/kg body weight and plasma half-life of 26 min.[11] In particular, a continuous infusion was chosen in order to avoid exenatide concentrations substantially above 0.3 nM, in the light of a possible biphasic dose-dependence suggested by experimental data.[9] Human serum albumin was added to the exenatide solution to avoid binding of the compound to the infusion material. Patients in the control group were treated with continuous infusion of saline and human serum albumin in equivalent velocities and durations. All randomized patients, including those meeting the angiographic exclusion criteria, were treated with the study drug for 6 h in order to evaluate any adverse effects.

In 100 consecutive patients, blood samples were collected at the end of the pPCI procedure in order to measure the exenatide plasma concentration in the patients randomized to exenatide treatment. Blood glucose was measured every 4 h until 6 h after the study infusion was terminated and serum amylase was collected during routine blood samples. Cardiac biomarkers (troponin T) were obtained before, immediately after, 6 h after, and 12–18 h after intervention.

## Study endpoints

The primary endpoint was the salvage index measured by CMR after 3 months. Secondary endpoints included final infarct size as assessed

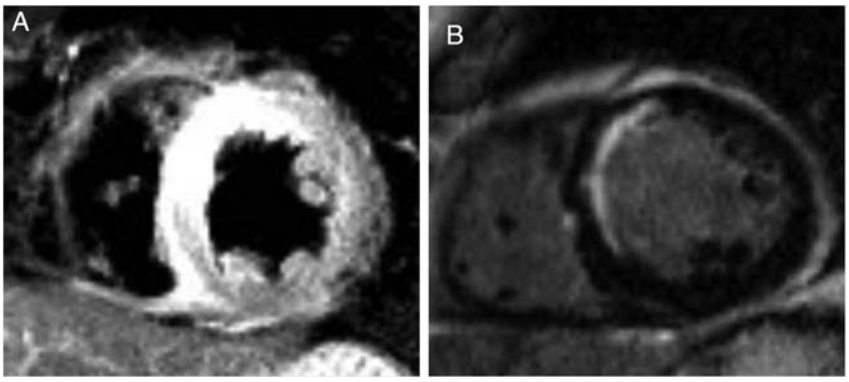


**Figure 1** Examples of T2-weighted and delayed enhancement images. A T2-weighted image used for area at risk analysis (*A*) and a delayed enhancement image used for final infarct size analysis (*B*) taken from a patient with a lesion in the left anterior descending artery.

by CMR after 3 months, final infarct size (g)/AAR (g), peak plasma level of troponin T, left ventricular ejection fraction (LVEF) determined by CMR after 3 months, and 30-day clinical events (cardiac death, any myocardial infarction, any stent thrombosis, and stroke). Clinical data were obtained from the Danish Civil Registration System, hospital medical records, and a telephone interview.

## Cardiac magnetic resonance

In all randomized patients without contraindications for CMR, an initial scan was performed during the index admission (within 1 week after pPCI) to assess the myocardial AAR. This time window was chosen, because the AAR evaluated by T2-weighted CMR within these limits have been shown to be a valid measurement in humans admitted with STEMI.[12–14] A second scan was performed 90 ± 21 days later in order to assess the final infarct size in all randomized patients. CMR was performed on a 1.5 T scanner (Avanto scanner, Siemens, Erlangen, Germany, or Intera system, Philips, Best, The Netherlands).

## Myocardial area at risk and salvage index

The myocardial AAR was assessed on the first CMR scan as oedema using a T2-weighted short tau inversion-recovery sequence (*Figure 1A*).[15,16] Multiple slices in the short-axis image plan were acquired to cover the entire LV. The images were analysed by an observer blinded to the treatment sequence using an ARGUS post-processing tool (ARGUS, Siemens, Erlangen, Germany). The endocardial and epicardial borders were manually traced in each short-axis image and the LV mass was calculated without incorporation of the papillary muscles and slow flowing blood in the trabeculae. The AAR was defined as the hyperintense area on T2-weighted images. A myocardial area was regarded as hyperintense when the signal intensity was higher than 2 SD of the signal intensity in the normal myocardium. The signal intensity in the normal myocardium was determined by tracing an area of at least 10 pixels within the normal myocardium. Hypointense areas within the AAR were considered a part of the AAR. The salvage index was calculated as follows: [AAR (g) − infarct size (g)]/AAR (g). Several experienced CMR readers validated the method used to assess salvage index and intra- and interobserver variability were 0.00 ± 0.03 and 0.01 ± 0.04, respectively.

## Infarct size and left ventricular ejection fraction by cardiac magnetic resonance

The final infarct size was evaluated on the second scan with delayed-enhancement CMR (*Figure 1B*).[17,18] Images were obtained ~10 min after intravenous injection of 0.1 mmol/kg body weight gadolinium-diethylenetriamine pentaacetic acid (Gadovist, Bayer Schering, Berlin, Germany) as an ECG-triggered inversion-recovery sequence. In a single slice, the inversion time was adjusted to null the signal from the normal myocardium. Multiple slices in the short-axis image plan were acquired to cover the entire LV. The final infarct size was assessed using the freely available software Segment v1.8 (http://segment.heiberg.se).[19] The endocardial and the epicardial borders were manually traced in all short-axis images and the LV myocardial mass was calculated. Papillary muscles were considered as part of the LV cavity. The infarct size, defined as the hyperenhanced myocardium on the delayed-enhancement images, was determined by an automatic approach.[19] The infarct size was expressed in grams as well as in percentage of the total LV mass.

Left ventricular ejection fraction was assessed by CMR on an ECG-triggered balanced steady-state free procession cine sequence by applying multiple slices in the short-axis image plan covering the entire LV. On cine short-axis CMR images, the LV volume was calculated by manually tracing the endocardial borders in all 25 phases. The diastolic and the systolic frames were automatically identified according to the size of the LV blood pool area, and LVEF was calculated accordingly. Papillary muscles were included in the LV lumen. The analysis was performed with an ARGUS post-processing tool.

## Sample size and statistical analysis

Based on previous results of ischaemic postconditioning in patients with STEMI, we assumed the average salvage index measured by CMR to be 0.50 with a standard deviation of 0.16. With a 5% type 1 error risk and a power of 80%, inclusion of 40 patients in each group were needed to detect a 20% difference in salvage index. No data exist on the clinical relevant difference in salvage index; therefore, the 20% difference was chosen arbitrarily.

The following analyses were performed: (i) analysis of patients fulfilling inclusion and exclusion criteria with data for salvage index and final infarct size (per-protocol); and (2) intention-to-treat analysis of

secondary endpoints of patients fulfilling inclusion and exclusion criteria (intention-to-treat).

Categorical variables were compared with the $\chi^2$ test or Fisher's exact test. Continuous variables were compared using Student's *t* or Mann–Whitney's tests. The primary endpoint was adjusted for pre-infarction and collateral flow in an analysis of covariance, because these patients may have inadvertently been protected against reperfusion injury. To compare the relationship between the AAR and the infarct size, a regression analysis was performed, and an analysis of covariance was used to test for equality of the regression lines for the exenatide group and the placebo group. Possible interaction between variables was evaluated using a multivariable regression analysis with salvage index as the dependent variable. A two-sided *P*-value of $<0.05$ was considered statistically significant. All statistical analyses were performed with SPSS software version 17 (SPSS Inc., Chicago, IL, USA).

# Results

## Study population

*Figure 2* shows the trial profile. In the study period, a total number of 578 consecutive patients were admitted acutely to the catheterization laboratories and screened for inclusion, of whom 191 patients were not eligible for the following reasons: did not understand information content (43), did not met ECG criteria (29), duration of symptoms >12 h (31), unconsciousness (21), cardiogenic shock (16), study refusal (15), contraindication to perform CMR (12), other severe disease (12), and no reason reported (12). An additional 215 of the randomized patients were excluded after the angiography for the reasons shown in *Figure 2*. There was no statistically significant difference between treatment groups in terms of reasons for exclusion. A final number of 172 patients thus met the inclusion criteria and were eligible for the intention-to-treat analysis. Cardiac magnetic resonance scan was performed 3 months after pPCI in 117 patients (68% of the eligible patients). Reasons for not performing a CMR are shown in *Figure 2*. One hundred and five patients (61% of the eligible patients) were available for per-protocol study analysis of the primary endpoint.

*Table 1* shows the baseline demographics, angiographic, and procedural results for all randomized patients and for the patients included in the study. The treatment groups were well balanced, despite a trend towards a higher frequency of anterior infarcts in the placebo group. The AAR was not statistically different between treatment groups (*Table 2*).

Patients included in the study were slightly but significantly older than excluded patients (63 vs. 62 years; $P = 0.016$), otherwise there were no differences between the included and excluded patients in terms of baseline clinical variables. The comparison

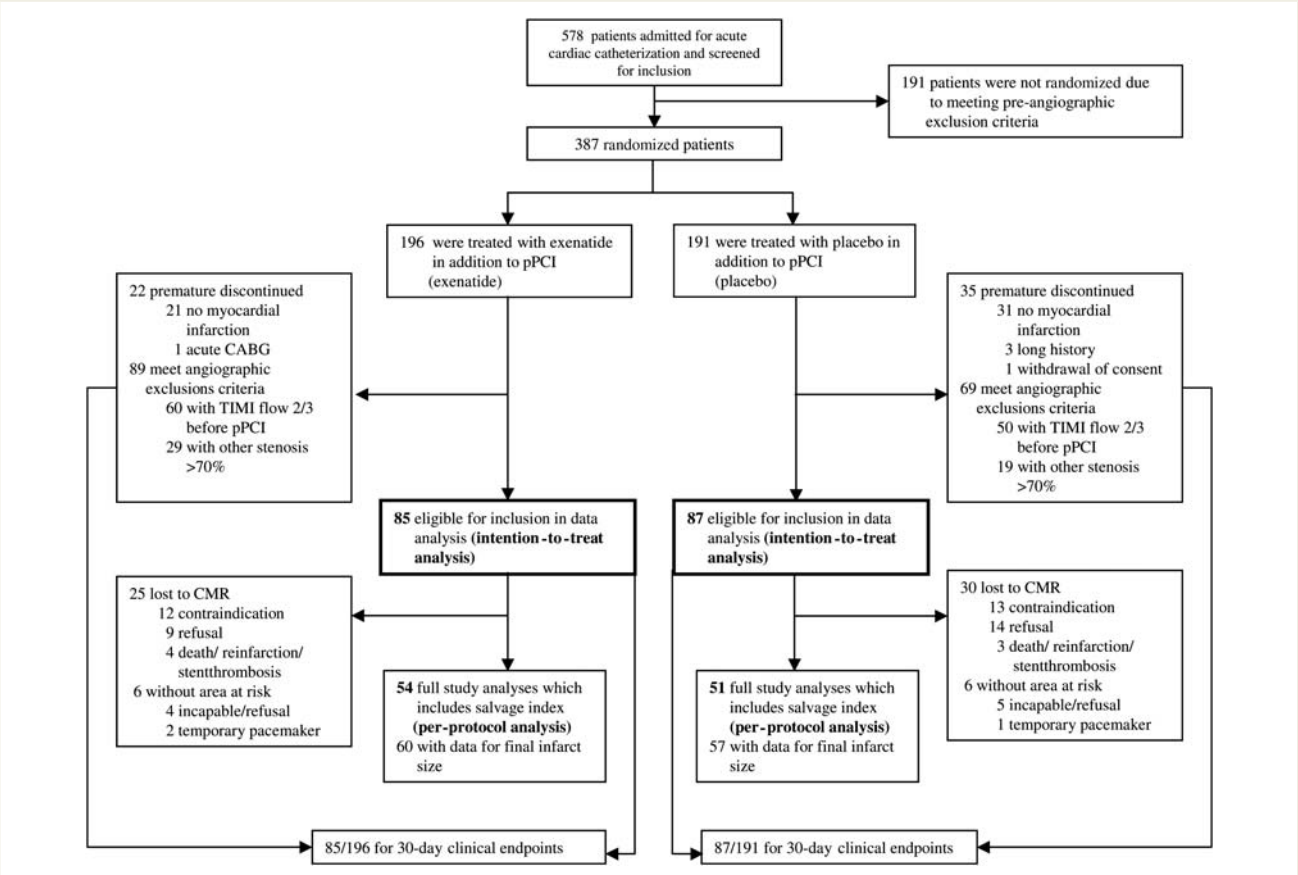


**Figure 2** Trial profile. CABG, coronary artery bypass graft operation; CMR, cardiac magnetic resonance; pPCI, primary percutaneous coronary intervention. TIMI; Thrombolysis in Myocardial Infarction.

**Table 1 Baseline clinical, angiographic, and procedural characteristics**

| | Included patients ($n = 172$) | | Randomized patients ($n = 387$) | |
|---|---|---|---|---|
| | Exenatide ($n = 85$) | Placebo ($n = 87$) | Exenatide ($n = 196$) | Placebo ($n = 191$) |
| Age (years) | 63 ± 10 | 63 ± 11 | 62 ± 11 | 62 ± 12 |
| Male | 66 (78) | 64 (74) | 160 (82) | 146 (76) |
| BMI (kg/m$^2$) | 27 ± 5 | 27 ± 4 | 27 ± 4 | 28 ± 4 |
| Diabetes mellitus | 3 (4) | 8 (9) | 14 (7) | 21 (11) |
| Family history of CAD | 39 (46) | 35 (40) | 80 (41) | 73 (38) |
| Current smokers | 25 (29) | 19 (22) | 49 (25) | 52 (27) |
| Hypertension | 25 (29) | 30 (35) | 73 (37) | 74 (39) |
| Hypercholesterolaemia | 37 (44) | 51 (58) | 88 (45) | 96 (50) |
| Previous PCI | 5 (6) | 11 (13) | 14 (7) | 17 (9) |
| Pre-infarct angina | 17 (20) | 13 (15) | 39 (20) | 26 (14) |
| Pre-infarct medical treatment | | | | |
| β-blockers | 6 (7) | 8 (9) | 16 (8) | 13 (7) |
| ACE inhibitors | 6 (7) | 6 (7) | 22 (11) | 21 (11) |
| Statin | 11 (13) | 21 (24) | 29 (15) | 34 (18) |
| Aspirin or clopidogrel | 7 (8) | 17 (20) | 24 (12) | 31 (16) |
| Blood glucose | 6 (5–7) | 7 (6–8) | 6 (5–7) | 7 (6–8) |
| Time to CMR 1 (days) | 1 (1–4) | 2 (1–6) | 2 (1–2) | 2 (1–3) |
| Time to CMR 2 (days) | 90 (80–96) | 92 (83–103) | 90 (84–95) | 90 (79–99) |
| Symptom-to-balloon time (min) | 175 (125–306) | 185 (123–275) | 180 (124–280) | 197 (130–280) |
| Anterior infarct | 30 (35) | 39 (45) | 68 (39) | 77 (48) |
| Collateral flow (Rentrop grade 2/3) | 13 (15) | 9 (10) | 30 (15) | 16 (8) |
| TIMI grade 3 after procedure | 79 (93) | 82 (94) | 184 (94) | 181 (95) |
| Thrombectomy | 55 (65) | 54 (62) | 100 (51) | 92 (48) |
| Stent implantation | 78 (92) | 76 (87) | 137 (70) | 145 (76) |
| Treatment with GP IIb/IIIa inhibitor | 78 (92) | 79 (91) | 155 (79) | 144 (70) |

Data are presented as mean ± SD, median (IQR), or *n* (%) unless otherwise indicated.
ACE, angiotensin-converting enzyme; BMI, body mass index; CAD, coronary artery disease; CMR, cardiac magnetic resonance; GP, glycoprotein; PCI, percutaneous coronary intervention.

between patients undergoing full study analysis and patients lost to imaging follow-up showed that more male patients underwent full study analysis (81 vs. 65%; $P = 0.018$) and that these patients had slightly lager body mass index (27 vs. 26; $P = 0.03$). Otherwise, no statistically significant differences were observed. In addition, the groups were comparable with regard to the peak troponin T level (6.4 ± 4.9 vs. 6.5 ± 5.4 μg/L; $P = 0.85$).

## Salvage index and infarct size

In the primary endpoint, we found a 15% larger salvage index in the exenatide group than in the placebo group (*Table 2*, $P = 0.003$). Correspondingly, the infarct size/AAR ratio was 23% smaller in the exenatide group ($P = 0.003$, *Table 2*). In a multivariable analysis adjusting for a history of pre-infarct angina, and angiographically detected collaterals, the difference in salvage index between treatment groups remained statistically significant ($P = 0.008$). In the regression analysis of infarct size plotted against the myocardial AAR, the line for the exenatide group lies significantly below the line for the placebo group ($P = 0.011$) (*Figure 3*), indicating that patients in the exenatide group develop significantly smaller infarcts for an equivalent AAR. In addition, there was a trend towards a smaller final infarct size expressed in grams in the exenatide group ($P = 0.11$) (*Table 2*). Eighty-one per cent of the patients had their initial CMR for evaluation of AAR done within 48 h. Analysing those patients alone did not change the results with a significant difference in salvage index between the exenatide group ($n = 46$) and the placebo group ($n = 39$) (0.72 ± 15 vs. 0.63 ± 11; $P = 0.002$).

In the patient subgroup with infarctions in the area supplied by the left anterior descending artery, the salvage index was 19% larger in the exenatide group than that of the placebo group ($P = 0.023$) and final infarct size/AAR ratio 30% smaller ($P = 0.024$) (*Table 2*). In these patients, the regression analysis of infarct size plotted against the myocardial AAR revealed a significant difference between the line for the exenatide group and the line for the placebo group ($P = 0.025$) (*Figure 3*). In the subgroup with non-anterior infarct location, we also found a trend towards a larger salvage index in the exenatide group ($P = 0.05$) (*Table 2*), but no difference in the regression analysis. There was, however, no interaction of infarct location with exenatide

**Table 2 Outcomes evaluated with cardiac magnetic resonance**

| | n | Exenatide | n | Placebo | P-value |
|---|---|---|---|---|---|
| Overall study population | | | | | |
| Salvage index[a] | 54 | 0.71 ± 0.13 | 51 | 0.62 ± 0.16 | 0.003 |
| Infarct size (g)/area at risk (g) | 54 | 0.30 ± 0.15 | 51 | 0.39 ± 0.15 | 0.003 |
| Area at risk (g) | 54 | 42 ± 21 | 51 | 39 ± 14 | 0.43 |
| Final infarct size (g) | 60 | 13 ± 9 | 57 | 17 ± 14 | 0.11 |
| Final infarct size (%LV) | 60 | 11 ± 7 | 57 | 12 ± 6 | 0.33 |
| LVEF 3 months (%) | 60 | 55 ± 9 | 57 | 55 ± 11 | 0.82 |
| Anterior infarct location[b] | | | | | |
| Salvage index[a] | 20 | 0.74 ± 0.11 | 21 | 0.62 ± 0.18 | 0.023 |
| Infarct size (g)/area at risk (g) | 20 | 0.27 ± 0.12 | 21 | 0.39 ± 0.19 | 0.024 |
| Area at risk (g) | 20 | 53 ± 24 | 21 | 45 ± 17 | 0.14 |
| Final infarct size (g) | 23 | 17 ± 11 | 25 | 21 ± 19 | 0.32 |
| Final infarct size (%LV) | 23 | 13 ± 9 | 25 | 14 ± 8 | 0.76 |
| LVEF 3 months (%) | 23 | 55 ± 11 | 25 | 51 ± 14 | 0.27 |
| Non-anterior infarct location[b] | | | | | |
| Salvage index[a] | 34 | 0.69 ± 0.13 | 30 | 0.63 ± 0.13 | 0.05 |
| Infarct size (g)/area at risk (g) | 34 | 0.32 ± 0.14 | 30 | 0.39 ± 0.13 | 0.05 |
| Area at risk (g) | 34 | 34 ± 11 | 30 | 35 ± 11 | 0.99 |
| Final infarct size (g) | 37 | 11 ± 7 | 32 | 14 ± 6 | 0.18 |
| Final infarct size (%LV) | 37 | 10 ± 6 | 32 | 11 ± 5 | 0.19 |
| LVEF 3 months (%) | 37 | 55 ± 9 | 32 | 58 ± 8 | 0.13 |

Data are presented as mean ± SD.
LV, left ventricle; LVEF, left ventricle ejection fraction.
[a]Salvage index: [area at risk (g) − infarct size (g)]/area at risk (g).
[b]For interaction of infarct location with exenatide treatment, $P = 0.58$ for salvage index and $P = 0.33$ for final infarct size (g).

treatment in terms of salvage index ($P = 0.58$) or infarct size (g) ($P = 0.33$).

## Other endpoints

The secondary endpoint of LVEF after 90 days was not significantly different between treatment groups (*Table 2*). In the intention-to-treat analysis, assessment of the secondary endpoint of peak troponin T levels showed no difference between the exenatide and placebo groups (6.7 ± 5.4 vs. 6.2 ± 5.4 μg/L; $P = 0.50$).

In the intention-to-treat analysis, no differences in the secondary endpoints of clinical events during the 30-day follow-up between the exenatide and placebo groups were seen (*Table 3*). Of the four patients who died during 30-day follow-up, four patients died during hospitalization, three due to pump failure (two treated with exenatide and one with placebo) and one patient treated with placebo died due to a definite stent thrombosis. The mean plasma concentration of exenatide was 0.177 ± 0.069 nmol/L (range from 0.097 to 0.391 nmol/L) (*Figure 4*). No episodes of hypoglycaemia, pancreatitis, or any other adverse effect were reported.

# Discussion

The present study is the first to show that treatment with the GLP-1 analogue exenatide administered at the time of reperfusion increases myocardial salvage in a population of STEMI patients with TIMI flow 0/1 treated with primary PCI.

It has previously been demonstrated that an increased salvage index is associated with an improved clinical outcome in patients with STEMI.[20] Whether the relative increase of 15% in our population as a whole and 19% in the anterior infarct subset following exenatide treatment will translate into an improved clinical outcome remains to be proven. In addition to this increase in salvage index and a corresponding decrease in infarct size compared with the AAR size, there was a trend towards a decrease in final infarct size. However, the study was not powered to show a significant difference in infarct size. With a difference of 4 g corresponding to a 24% reduction in infarct size (as observed in the present study) and standard deviation of 11 g, a total of 119 patients would be needed in each treatment group in order to detect a statistically significant difference. The regression analysis of infarct size vs. AAR showed significant correlation between these two parameters in both groups and in addition showed that datapoints for the exenatide group were below datapoints for the control group. Importantly, we found no influence of a history of pre-infarction angina or well-developed collateral blood supply to the occluded vessel territory on the outcome.

The assessment of the efficacy of a novel cardioprotective strategy requires the measurement of both infarct size and AAR.[21] Both infarct size and AAR were measured using CMR. In terms of infarct

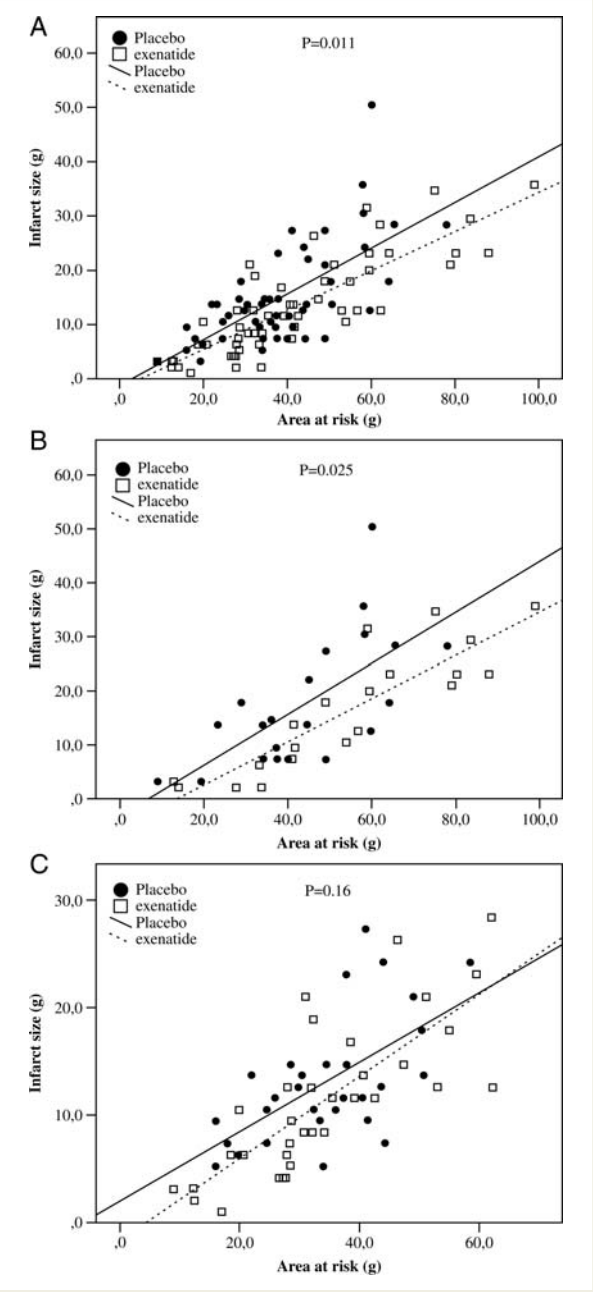


**Figure 3** Infarct size plotted against area at risk. Infarct size (g) was plotted against the myocardial area at risk (g) measured by T2-weighted cardiac magnetic resonance techniques. (*A*) In the per-protocol analysed population ($n = 105$), the line for the exenatide group lies significantly below the line for the placebo group ($P = 0.011$). In both groups, the infarct size correlates with the area at risk ($r = 0.83$ and 0.67, $P < 0.001$). (*B*) In the subgroup of patients with left anterior descendent artery infarcts ($n = 41$), the line for the exenatide group lies significantly below the line for the placebo group ($P = 0.025$). In both groups, the infarct size correlates with the area at risk ($r = 0.88$ and 0.67, $P < 0.001$). (*C*) Patients with non-anterior infarcts ($n = 64$). The lines were not significantly different ($P = 0.16$). In both groups, the infarct size correlates with the area at risk ($r = 0.79$ and 0.61, $P < 0.001$).

size, CMR is superior to single photon emission computed tomography with regard to the detection of myocardial infarction and reproducibility of infarct size.[22,23] Furthermore, AAR measured using CMR has been validated against histopathological,[24] and single photon emission computed tomography measurements of AAR.[15] In an animal study, validation of AAR existed for no more than 2 days.[24] In contrast, several human studies have shown oedema by CMR to persist unchanged up to 7 days.[12–14] However, due to this discrepancy, we also performed analysis of salvage index in the subgroup of patients with the AAR measurement performed within 48 h (81% of patients). In these patients, the difference between treatment groups did still remain significant and the increase in salvage index with exenatide was equal to the effect observed in the total study population. Furthermore, concerns regarding the use of T2-weighted imaging to assess AAR have recently been raised, because it is speculated that the novel treatment in itself may reduce the oedema and hence reduce the size of AAR.[21] However, if this hold true, it is important to emphasize that such an effect on AAR would underestimate the favourable effect of exenatide on salvage index.

It was previously demonstrated that intravenous infusion of GLP-1 commenced 4 h after pPCI resulted in an increase in LVEF.[25] In the present study, we could not detect any exenatide-induced improvement in LVEF. It should, however, be emphasized that the former study did not address cardioprotection during reperfusion, because it is generally accepted that reperfusion injury only occurs in the first minutes after restoration of blood flow.[26] In addition, LVEF is a relatively gross measure of the post-infarction damage, partly due to compensatory hyperkinesias of the non-infarcted myocardium contributing to preserve the global LVEF despite regional dysfunction.[27]

In STEMI patients with a symptom-to-balloon duration between 180 and 240 min and current pre-hospital medical treatment, between 30 and 40% of the index vessels have a normal or near-normal angiographic flow at admission.[28,29] In the present study, we excluded patients with TIMI flow 2 and 3 in the infarct-related artery before intervention. Accordingly, patients who achieved reperfusion prior to intervention were assumed not to have any cardioprotective effect of exenatide. It is, however, reasonable to suggest that administration of exenatide pre-hospitally may exert an effect in patients who undergo reperfusion before admission.

In our study, a continuous infusion was chosen, because we aimed to obtain plasma exenatide concentrations within the effective range suggested by our animal experiments (0.03–0.30 nmol/L) and avoiding too high plasma concentrations (3.0 nmol/L) possibly leading to loss of cardioprotection as previously suggested.[9] Accordingly, measurements of the actual exenatide plasma concentration in a sample of 50 patients indicate that the treated patients reached concentrations at the end of the procedure in a satisfactory agreement with protocol goals. However, a more recent study on pigs found exenatide to be cardioprotective using a combination of bolus intravenous and bolus subcutaneous administration prior to reperfusion and twice daily subcutaneous regimen for the following 3 days.[10] The latter study may be more relevant for the translation into clinical settings. Unfortunately, this study was not published when the present study commenced and no plasma exenatide concentrations were

**Table 3 Clinical outcome 30 days after initial treatment**

| | Included patients ($n$ = 172) | | | Randomized patients ($n$ = 387) | | |
|---|---|---|---|---|---|---|
| | Exenatide ($n$ = 85) | Placebo ($n$ = 87) | $P$-value | Exenatide ($n$ = 196) | Placebo ($n$ = 191) | $P$-value |
| Cardiac death | 2 (2) | 2 (2) | 1 | 4 (2) | 4 (2) | 0.91 |
| Myocardial infarction | 1 (1) | 0 (0) | 1 | 1 (1) | 1 (1) | 0.95 |
| Stent thrombosis | 0 (0) | 1 (1) | 1 | 0 (0) | 1 (1) | 0.51 |
| Stroke | 1 (1) | 1 (1) | 1 | 1 (1) | 1 (1) | 0.95 |
| Total events[a] | 4 (5) | 4 (5) | 1 | 6 (3) | 7 (4) | 0.67 |

Data are presented as number of events (%).
[a]Total events: cardiac death/myocardial infarction/stent thrombosis/stroke.

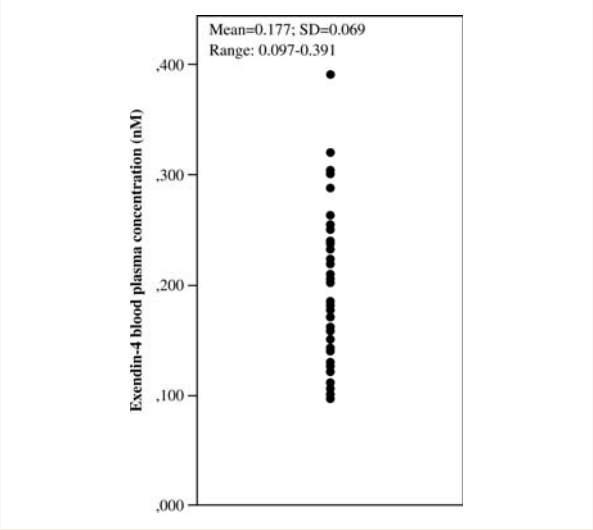


**Figure 4** Exenatide plasma concentration. This figure shows plasma concentrations of exendin-4 (the active component of exenatide) collected from 50 patients, who were randomized to exenatide. The blood sample was collected from the femoral sheath at the end of the procedure.

reported in this study. Thus, although the present treatment algorithm showed exenatide to be effective, future studies should focus on the titration of the optimal treatment dosing and timing of the treatment.

Ischaemic postconditioning,[29,30] cyclosporine,[31] and remote ischaemic conditioning[32] all limit reperfusion injury with a potentially favourable outcome in clinical settings. The results of our study suggest that exenatide can be added to the list of promising cardioprotective agents. Ischaemic postconditioning with consecutive occlusions using a balloon catheter after primary opening of the index vessel carries a potential risk of damaging the vessel or induces peripheral embolization. Exenatide, cyclosporine treatment, and remote ischaemic conditioning can all be carried out in STEMI patients treated with fibrinolysis, provided the treatment is initiated prior to reperfusion.

The mechanism of exenatide-mediated protection against reperfusion injury remains to be fully clarified. Two key phenomena in reperfusion injury do appear to be loss of mitochondrial integrity,[33] and myocyte hypercontracture associated with sarcolemmal rupture,[34] and a large body of experimental research suggests that reperfusion injury may be ameliorated by activation of a receptor-mediated survival pathway.[35] This pathway has previously been shown to be a target for GLP-1-mediated cardioprotection through activated of phosphoinositide 3 kinase.[36] However, other possible targets for exenatide have been identified such as increased glucose uptake, inhibition of apoptotic factors, and activation of cAMP and cGMP.[7] Thus, exenatide, acting on GLP-1 receptors, may exert its cardioprotective actions through a number of pathways encompassing metabolic, contractility, and antiapoptotic effects.

Only 60% of patients eligible for intention-to-treat analysis underwent two CMR scans and were thus eligible for per-protocol analysis. This induces a certain risk of selection bias. However, comparisons between per-protocol analysed patients and those lost to imaging follow-up did not indicate that a selection bias was introduced. Furthermore, 13% of the patients in the present study were excluded due to no myocardial infarction; however, this is in accordance with the incidence reported in a previous study.[37] Finally, the relatively small sample size did not allow for detection of any possibly infrequent adverse events of exenatide administration.

# Conclusion

Exenatide administered prior to reperfusion increased myocardial salvage in patients with STEMI and was not associated with adverse effects.

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