Tight Glycemic Control Reduces Heart Inflammation and Remodeling During Acute Myocardial Infarction in Hyperglycemic Patients

Raffaele Marfella, MD, PhD,* Clara Di Filippo, PhD,† Michele Portoghese, MD,§ Franca Ferraraccio, MD,♭ Maria Rosaria Rizzo, MD, PhD,* Mario Siniscalchi, MD, PhD,¶ Emilio Musacchio, MD,‖ Michele D’Amico, PhD,† Francesco Rossi, MD, PhD,† Giuseppe Paolisso, MD, PhD*

Naples, Sassari, Agropoli, and Campobasso, Italy

Objectives
We analyzed the molecular mechanisms evoked by tight glycemic control during post-infarction remodeling in human hearts.

Background
The molecular mechanisms by which tight glycemic control improves heart remodeling during acute myocardial infarction (AMI) are still largely unknown.

Methods
Eighty-eight patients with first AMI undergoing coronary bypass surgery were studied: 38 normoglycemic patients served as the control group; hyperglycemic patients (glucose ≥140 mg/dl) were randomized to intensive glycemic control (IGC) (n = 25; glucose 80 to 140 mg/dl) or conventional glycemic control (CGC) (n = 25; glucose 180 to 200 mg/dl) for almost 3 days before surgery, with insulin infusion followed by subcutaneous insulin treatment. Echocardiographic parameters were investigated at admission and after treatment period. During surgery, oxidative stress (nitrotyrosine, superoxide anion [O2–] production, inducible nitric oxide synthase [iNOS]), inflammation (nuclear factor kappa B [NFkB], tumor necrosis factor [TNF]-α), and apoptosis (caspase-3) were analyzed in biopsy specimens taken from the peri-infarcted area.

Results
Compared with normoglycemic patients, hyperglycemic patients had higher myocardial performance index (MPI) (p < 0.05), reduced ejection fraction (p < 0.05), more nitrotyrosine, iNOS, and O2– production, more macrophages, T-lymphocytes, and HLA-DR (Dako, Milan, Italy) cells, and more NFkB-activity, TNF-α, and caspase-3 levels (p < 0.01) in peri-infarcted specimens. After the treatment period, plasma glucose reduction was greater in the IGC than in the CGC group (p < 0.001). Compared with IGC patients, CGC patients had higher MPI (p < 0.02), had lower ejection fraction (p < 0.05), and had more markers of oxidative stress, more inflammation and apoptosis (p < 0.01) in peri-infarcted specimens.

Conclusions
Tight glycemic control, by reducing oxidative stress and inflammation, might reduce apoptosis in peri-infarcted areas and remodeling in AMI patients. (J Am Coll Cardiol 2009;53:1425–36) © 2009 by the American College of Cardiology Foundation

An unusually high prevalence of glycosuria in nondiabetic patients who have acute myocardial infarction (AMI) was noted as early as 1931 (1). Stress hyperglycemia during AMI is associated with an increased risk of in-hospital mortality in patients with and without diabetes (2). Moreover, 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. Nonetheless, the question of whether hyperglycemia is a mediator or marker of adverse outcomes remains unclear. Recommendations are being developed for strict glucose management in all hospitalized patients; however, glucose measurement has not been included in acute coronary syndrome risk indexes, and current guidelines do not suggest specific therapeutic targets for
glucose control (4). This relative lack of guidance concerning the risk management of patients with AMI and hyperglycemia might be because many aspects of the relation between glucose control and pathogenetic mechanisms responsible for favorable outcomes in patients with AMI have not been adequately defined. This gap in knowledge might limit treatment of elevated glucose levels in hospitalized patients with AMI. However, an active inflammatory infiltrate in the peri-infarct area and in unaffected viable myocardium has been described in patients with AMI (5). The inflammatory burden in the peri-infarct region is associated with worse short- and mid-term outcomes. This is not surprising, because the inflammatory response in this region probably amplifies myocardial remodeling. Cytokines such as tumor necrosis factor (TNF)-α are elaborated soon after myocardial ischemic injury and can acutely regulate myocyte survival or apoptosis, leading to acute cardiac remodeling, paving the way for heart failure (5). In this context, we have shown that hyperglycemic stress during AMI is associated with increased levels of some inflammatory markers, including C-reactive protein and interleukin-18 (6). These results fit with animal studies showing increased levels of peroxynitrite (an index of oxidative stress) and pro-inflammatory cytokines in the ischemic heart tissue of hyperglycemic mice. Both inflammation and oxidative stress correlated strictly with the glucose levels, leading to myocardial apoptosis and greater infarct size (7). These studies suggest that hyperglycemia amplifying oxidative stress and the inflammatory responses to myocardial ischemia might affect the prognosis of patients presenting with AMI. Nevertheless, whether strict glycemic control contributes to improve the early outcome of AMI in hyperglycemic patients has been recently debated (4). In this context, our study evaluates whether tight glycemic control during AMI would reduce myocardial levels of peroxynitrite, TNF-α, and apoptosis in myocardial biopsies obtained from patients with and without hyperglycemia who were admitted to emergency wards for first AMI and had to undergo coronary artery bypass graft surgery (CABG). We also sought to determine whether the early beneficial effects of tight glycemic control would result in improved myocardial performance as well as in a decreased incidence of acute complications.

Methods

Patients. The study group comprised 88 consecutive patients presenting with their first uncomplicated AMI within 3 to 8 h after the onset of pain; they were all admitted into the emergency wards of the Sassari and Campobasso Hospitals from January 2001 to June 2008. We defined AMI according to criteria recommended by the American College of Cardiology (8). Thus, patients were diagnosed as having an AMI if they had 2 values of serum troponin I >2.50 μg/l together with either typical symptoms of or electrocardiogram changes indicating acute ischemia. The eligibility criteria for the study were: 1) evidence of AMI within the last 8 h (troponin-I >2.50 μg/l or electrographic criteria of ST-segment modification); 2) first uncomplicated AMI; and 3) the need for CABG (8). Patients without ST-segment elevation myocardial infarction (NSTEMI) had an angiogram after admission and did not qualify for percutaneous coronary intervention and were therefore sent for CABG. The subjects with ST-segment elevation myocardial infarction (STEMI) received thrombolytic drugs (tissue plasminogen activator, 15 mg in bolus, 0.75 mg/kg for 30 min, or 0.50 mg/kg for 60 min; or tenecteplase 0.53 mg/kg), did not have ST-segment resolution, and thereafter went for angiogram, after which it was decided to perform CABG. Patients with previous AMI, inflammatory disorders, malignancy, renal diseases, or infection were not eligible for the study. Routine analyses were obtained on admission before coronary angiography and the start of full medical therapy, including beta-blocker drugs and/or calcium antagonists, low-dosage aspirin, nitrates, and heparin, as well as after achieved glycemic control goal for almost 3 days before CABG. Venous blood for troponin I (Behring Diagnostics, Westwood, Massachusetts) (normal values <2.0 μg/l) levels was collected in ethylenediaminetetraacetic acid-coated tubes immediately after patients arrived at the emergency department. Troponin I was measured with an Opus Magnum device (Behring Diagnostics). A discriminator value of 2.0 μg/l was used for troponin I, as recommended by the manufacturer. 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). Patients with hyperglycemia were subdivided into those treated with intensive glucose control (IGC group) and those treated with conventional glucose control (CGC group). The investigation conforms to the principles outlined in the Declaration of Helsinki for use of human tissue or subjects. The study protocol was approved by the institutional ethics committee at Sassari and Agropoli Hospitals. Written informed consent was obtained from all patients.

Study design. The study design was structured on the basis of protocol Yale (9). Upon emergency wards admission, hyperglycemic patients were randomly assigned to IGC or CGC. In patients with STEMI the insulin infusion was started after thrombolysis. In the patients in the CGC group, continuous insulin infusion of 50 IU Actrapid HM (Novo Nordisk, Rome, Italy) in 50 ml sodium chloride...
(0.9% with a Perfusor-FM pump [B. Braun, Melsungen, Germany]) was started only when blood glucose levels exceeded 200 mg/dl and was adjusted to keep blood glucose between 180 and 200 mg/dl. When blood glucose fell <180 mg/dl, insulin infusion was tapered and eventually stopped. In the IGC group, insulin infusion was started when blood glucose levels exceeded 140 mg/dl and adjusted to maintain glycemia at 80 to 140 mg/dl. During insulin infusion, oral feeding was stopped and parenteral nutrition (13 ± 5 kcal/kg \(1/2\)day\(^{-1}\)) was started. After the start of insulin infusion protocol, a glycemic control was provided every hour to obtain 3 consecutive values that were within the goal range. Capillary glucose levels were measured by fingerstick testing. Additionally, plasma glucose levels were checked every 2 h in both CGC and IGC patients throughout the study period. Measurements were not statistically different (data not shown). The infusion lasted until the stable glycemic goal (IGC group: 80 to 140 mg/dl; CGC group: 180 to 200 mg/dl) was maintained for at least 24 h. After the glycemic goal was maintained for 24 h, a parenteral nutrition was stopped and feeding was started according to European guidelines (10). Subcutaneous insulin was initiated at the cessation of the infusion. Insulin was given as short-acting insulin before meals and intermediate long-acting insulin in the evening, in both groups. In the IGC group, the treatment goal was a fasting blood glucose level of 90 to 140 mg/dl and a non-fasting level of <180 mg/dl (4). In the CGC group, the treatment goal was fasting blood glucose and postprandial levels of <200 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 according to evidence-based international guidelines for AMI (8).

**Echocardiographic assessment.** Patients enrolled in the study underwent 2-dimensional echocardiography at admission before starting full medical therapy as well as after achieved glycemic control goal for almost 3 days, before surgery. The study was performed with a standardized protocol and phased-array echocardiographs with M-mode, 2-dimensional, and pulsed, continuous-wave, and color flow Doppler capabilities. The ejection fraction was calculated from area measurements with the area-length method applied to the average apical area (11). The left ventricular internal dimension and interventricular septal were measured at the end diastole and end systole, and the wall motion score index was calculated according to American Society of Echocardiography recommendations (11). Isovolumetric relaxation time (IRT) was the time interval from cessation of left ventricular outflow to onset of mitral inflow, the ejection time (ET) was the time interval between the onset and the cessation of left ventricular outflow, and the mitral early diastolic flow deceleration time was the time interval between the peak early diastolic flow velocity and the end of the early diastolic flow. The total systolic time interval was measured from the cessation of 1 mitral flow to the beginning of the following mitral inflow. Isovolumetric contracting time was calculated by subtracting ET and IRT from the total systolic time interval. The ratio of velocity time intervals of mitral early and late diastolic flows (Ev/Avti) was calculated. The myocardial performance index (MPI) was calculated as: (IRT + isovolumetric contracting time)/ET.

**Biopsy of myocardium.** All patients underwent CABG after glucose goal was maintained for almost 3 days. After induction of anesthesia and median sternotomy, the heart of each patient was examined, and 3-mm partial-thickness biopsy specimens were taken from the peri-infarcted area. The infarcted zone was identified as a yellow area surrounded by a reddish purple band of granulation tissue or as gray area with fine yellow lines at its periphery. The peri-infarct zone was identified as a zone immediately adjacent the zone with the anatomical characteristics of myocardial infarct. Moreover, transesophageal echocardiogram was performed to evaluate the peri-infarct zone. All biopsies were performed before CABG, during ventilation with a fraction of inspired oxygen of 40% and peripheral oxygen saturations of >95%.

**ANALYSIS OF SPECIMENS.** One-half of each biopsy specimen was fixed in formalin, sectioned to a thickness of 5 µm, mounted on slides, and stained with hematoxylin and eosin. The mounted specimens were then examined for evidence of ischemia or kept for immunohistochemistry. The other one-half of the specimen was frozen in liquid nitrogen for the after enzyme-linked immunosorbent assay (ELISA) analysis.

**Immunohistochemistry.** Serial sections were incubated with specific antibodies anti-CD3, HLA-DR, and anti-CD68 (Dako, Milan, Italy); caspase-3, anti-inducible nitric oxide synthase (iNOS), antymyoglobin (Santa Cruz Biotechnology, Santa Cruz, California); antiprotein kinase C, anti–TNF-α (R&D Systems, Milan, Italy). Specific antibodies that selectively recognize the activated form of nuclear factor kappa B (NFκB) p65 and p50 subunits (Santa Cruz Biotechnology) were used. Nitrotyrosine was determined by antinitrotyrosine rabbit polyclonal antibody (DBA, Milan, Italy). The iNOS quantization was scored for intensity of immunostaining. For the quantitative analysis for histology, we determined the CD3, HLA-DR, and CD68-positive–rich areas (Dako). Analysis of experiments was performed with a PC-based 24-bit color image-analysis system (IM500, Leica Microsystems AG, Milan, Italy) as previously described (12). The percentage of the total area with positive color for each section was recorded. The specimens were analyzed by an expert pathologist (intraobserver variability 6%) blinded to the patient's diagnosis.

**Biochemical assays.** Myocardial tissues were lysed and centrifuged for 10 min at 10,000 g at 4°C. After centrifugation, TNF-α levels were quantified in heart tissue with specific ELISA kits (Santa Cruz Biotechnology; R&D Systems; Imgenex, San Diego, California). Nuclear extracts from heart specimens were obtained as described by Ohls-
son et al. (13). We used a specific antibody that selectively recognizes the activated form of the NFκB p65 and p50 subunits (Active Motif Europe, Rixensart, Belgium). Nitrotyrosine was assayed in heart tissue with kits supplied by Hycult Biotech (Uden, the Netherlands). Production of superoxide anion (\(O_2^-\)) was measured as the superoxide dismutase-inhibitable reduction of cytochrome c, as previously described (12). Cardiac cell apoptosis was assessed by caspase-3 as previously described (7).

**Statistical analysis.** Data are presented as mean ± SD. Continuous variables were compared among the groups of patients with 1-way analysis of variance for normally distributed data and Kruskal-Wallis test for non-normally distributed data. Moreover, Fisher exact test was performed for nominal variables. The Kolmogorov-Smirnov test was used to assess whether continuous variables were normally distributed or not. We compared data with a Kruskal-Wallis test for triglycerides and troponin. When differences were found among the groups, Bonferroni correction was used to make pairwise comparisons. General linear model repeated measures analysis was used to evaluate differences in each group on admission and before surgery. To investigate the independent contribution of glycemic levels in the modification of TNF-α and echocardiographic parameters, a multiple regression analysis was performed. A value of \(p \leq 0.05\) was considered statistically significant. All calculations were performed with the computer program SPSS version 12 (SPSS Inc., Chicago, Illinois).

**Results**

**Baseline characteristics of the patients on admission at emergency wards.** Of the 88 study patients, 38 (43%) had admission glucose measurements <140 mg/dl. Among the patients with glycemic levels >140 mg/dl, 25 were randomly assigned to the CGC group and 25 were assigned to the IGC group. There were no differences in the mean age, body mass index, sex distribution, smoking habits, levels of plasma cholesterol, and triglycerides among the 3 groups. The use of diuretic drugs, angiotensin-converting enzyme inhibitor drugs, beta-blocker drugs, and calcium channel-blocker therapy was similar in both groups (Table 1). The AMI therapy was similar in both groups (data not shown). The thrombolysis was performed in all patients presenting the electrocardiogram with STEMI: 22 patients from the control group, 14 patients from the CGC group, and 15 patients from the IGC group. In these patients, after 1 h from thrombolysis, there was not any change in ST-segment. The site of AMI and the time of reperfusion of the patients with STEMI were not different between the groups (data not shown). The admission plasma glucose level in IGC patients (207.6 ± 16.6 mg/dl) was higher than that in the CGC group (201.9 ± 22.2 mg/dl), but the differences were not statistically significant. 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 onset of symptoms and the admission troponin I levels (\(R^2 = 0.005, p = 1.00\)). Hyperglycemic patients, both STEMI and NSTEMI patients, had higher infarct segment length (\(p < 0.05\)) and wall motion scores (\(p < 0.01\)) but lower ejection fraction (\(p < 0.05\)) than normoglycemic patients. Moreover, they had an increased MPI (\(p < 0.02\)) and reduced transmural Doppler flow (\(p < 0.05\)) and pulmonary venous flow analysis (\(p < 0.02\)). There were no differences between IGC and CGC groups (Table 1). Finally, among the 3 groups, we have not shown statistically significant differences in echocardiographic parameters of STEMI and NSTEMI patients (IGC group: infarct segment length, STEMI 39.2 ± 2.6% vs. NSTEMI 38.3 ± 1.9%, \(p = 0.274\) – ejection fraction, STEMI 35.9 ± 2.4% vs. NSTEMI 37.4 ± 2.9%, \(p = 0.165\); CGC group: infarct segment length, STEMI 38.4 ± 2.2% vs. NSTEMI 37.4 ± 2.8%, \(p = 0.328\) – ejection fraction, STEMI 36.9 ± 3.1% vs. NSTEMI 37.2 ± 3.2%, \(p = 0.817\); control group: infarct segment length, STEMI 32.1 ± 2.6% vs. NSTEMI 30.7 ± 1.7%, \(p = 0.207\) – ejection fraction, STEMI 42.9 ± 3.4% vs. NSTEMI 44.6 ± 3% , \(p = 0.213\)).

**Glucose-lowering treatment and echocardiographic parameters.** The adherence to the infusion protocol was high in CGC (\(n = 23\); 92%) and IGC (\(n = 24\); 96%) groups. In both groups, the mean time required to achieve blood glucose target was 7 ± 1.7 h, and the subsequent mean duration of the insulin infusion was 38.3 ± 5.7 h. After insulin infusion, multidose insulin (3 or more daily doses) was used in all patients in both groups. The treatment duration was 5.8 ± 0.9 days for both groups without significant differences between CGC and IGC groups (Table 2). Further details on nutritional intake and insulin doses are shown in Table 2. There were no 6-day mortalities in either group. Both control group and IGC-treated patients had a lower incidence of atrial fibrillation (\(p < 0.01\)) and had a lower incidence of pneumonia (\(p < 0.01\)) (Table 2). After the treatment period, plasma glucose reduction was greater in the IGC group than in the CGC group (78 ± 21 mg/dl vs. 10 ± 15 mg/dl; \(p < 0.001\)). Blood glucose <70 mg/dl with and without symptoms was more frequent during the initial 24 h in IGC group (32%) than in CGC group (12%). After the treatment period, CGC patients had higher infarct segment length (\(p < 0.05\)), MPI (\(p < 0.02\)), and wall motion scores (\(p < 0.01\)) but lower ejection fraction (\(p < 0.05\)) than IGC patients. There were no differences in cholesterol and triglycerides levels after intervention study among the groups (Table 3).

**Surgery procedures.** There was no difference in the coronary vessels with >50% stenosis (3.18 ± 0.06 control group; 3.21 ± 0.08 CGC group; 3.19 ± 0.04 IGC group, \(p = NS\)) or the number of vessels bypassed (3.13 ± 0.04 control group; 3.11 ± 0.09 CGC group; 3.19 ± 0.09 IGC group, \(p = NS\)). Cross-clamp (46.9 ± 1.1 min control group; 45.5 ± 1.2 min CGC group; 44.6 ± 1.8
Significantly intense immunostaining and GLYCEMIC CONTROL REDUCES OXIDATIVE STRESS IN THE ISCH-peri-infarcted tissue compared with normoglycemic tissue protein levels of iNOS were present in hyperglycemic

Data are presented as mean ± SD, % (n), or median (interquartile range) according to their distribution. *p < 0.05 versus control group.

ACE = angiotensin-converting enzyme; BMI = body mass index; CGC = conventional glycemic control; ECG = electrocardiographic; HDL = high-density lipoprotein; IGC = intensive glycemic control; PVF = pulmonary venous flow; PVF has been found to contain both a systolic(s) and diastolic(d) forward phase and a reverse phase during atrial contraction.

min IGC group, p = NS) and cardiopulmonary bypass times (92.6 ± 2.9 min control group; 91.5 ± 2.6 min CGC group; 93.7 ± 3.1 min IGC group, p = NS) were also similar between groups.

Analysis of peri-infarcted myocardial specimens. TIGHT GLYCEMIC CONTROL REDUCES OXIDATIVE STRESS IN THE ISCHEMIC HEART. Significantly intense immunostaining and protein levels of iNOS were present in hyperglycemic peri-infarcted tissue compared with normoglycemic tissue (p < 0.001). In hyperglycemic patients, iNOS levels were more abundant in ischemic specimens from the CGC group compared with the IGC group (p < 0.001) (Fig. 1). Notably, iNOS expression in peri-infarcted area was strongly dependent on glycemic control, as also reflected by the statistically significant correlation (R = 0.42, p < 0.01) between mean plasma glucose levels during the treatment period and iNOS expression. Significantly higher levels of O$_2^-$ were present in ischemic tissue from hyperglycemic
patients compared with ischemic tissue from normoglycemic patients ($p < 0.001$). In hyperglycemic patients, significantly higher levels of $O_2^\cdot$ were present in peri-infarcted area from CGC patients compared with tissue of peri-infarcted area from IGC patients ($p < 0.001$) (Fig. 1). Moreover, $O_2^\cdot$ levels in ischemic specimens were strongly dependent on glycemic control, as also reflected by the statistically significant correlation between mean plasma glucose levels during the treatment period and nitrotyrosine concentrations ($R = 0.52, p < 0.001$). Finally, we have not shown any correlation between the total amount of insulin administered (IU/kg) as well as the amount of insulin infused and the reduction of cardiac nitrotyrosine levels ($R = 0.02, p < 0.4$; $R = 0.01, p < 0.1$).

**TIGHT GLYCEMIC CONTROL REDUCES INFLAMMATION IN THE ISCHEMIC HEART.** Compared with normoglycemic patients, hyperglycemic patients all had a significantly greater portion of peri-infarcted area occupied by macrophages ($p < 0.01$) and T-lymphocytes ($p < 0.01$) as well as greater expression of HLA-DR (Dako) antigen ($p < 0.01$). Compared with the CGC group, the IGC group presented with a significantly smaller portion of peri-infarcted area occupied by macrophages ($p < 0.01$) and T-lymphocytes ($p < 0.01$) as well as lower expression of HLA-DR (Dako) ($p < 0.01$)

### Table 2
**Insulin Therapy, Nutrition, and Early AMI Complications**

<table>
<thead>
<tr>
<th></th>
<th>IGC Group</th>
<th>CGC Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>24</td>
<td>23</td>
<td>38</td>
</tr>
<tr>
<td>Treatment period time, days</td>
<td>5.8 ± 0.9</td>
<td>5.8 ± 1.0</td>
<td>—</td>
</tr>
<tr>
<td>Insulin infusion time, h</td>
<td>38.5 ± 5.7</td>
<td>38.2 ± 5.9</td>
<td>—</td>
</tr>
<tr>
<td>Mean 24-h glucose values after admission, mg/dl</td>
<td>162.7 ± 22.6*</td>
<td>192.4 ± 21.5.</td>
<td>—</td>
</tr>
<tr>
<td>Achievement time of blood glucose target, h</td>
<td>7.2 ± 1.6</td>
<td>6.9 ± 1.8</td>
<td>—</td>
</tr>
<tr>
<td>Time of blood glucose target, days</td>
<td>4.2 ± 0.9</td>
<td>4.2 ± 0.8</td>
<td>—</td>
</tr>
<tr>
<td>Total amount of feeding (kcal/kg–1/day–1)</td>
<td>15 ± 6</td>
<td>15 ± 7</td>
<td>—</td>
</tr>
<tr>
<td>Amount of parenteral calories (kcal/kg–1/day–1)</td>
<td>13 ± 5</td>
<td>13 ± 5</td>
<td>—</td>
</tr>
<tr>
<td>Amount of insulin infused (IU/day)</td>
<td>76 ± 11*</td>
<td>29 ± 4</td>
<td>—</td>
</tr>
<tr>
<td>Daily subcutaneous insulin dose (IU/day)</td>
<td>51 ± 12*</td>
<td>29 ± 11</td>
<td>—</td>
</tr>
<tr>
<td>Atrial fibrillation, n (%)</td>
<td>(12)**†</td>
<td>7 (30)</td>
<td>2 (3)*</td>
</tr>
<tr>
<td>Infections (pneumonia and wound), n (%)</td>
<td>0 (0)*</td>
<td>6 (26)</td>
<td>0 (0)*</td>
</tr>
<tr>
<td>Heart failure, n (%)</td>
<td>3 (12)**†</td>
<td>7 (30)</td>
<td>2 (3)*</td>
</tr>
</tbody>
</table>

Data are mean ± SD unless otherwise indicated. *p < 0.05 versus conventional glycemic control (CGC) group; †p < 0.05 versus control group. AMI = acute myocardial infarction; IGC = intensive glycemic control.

### Table 3
**Glucose-Lowering Treatment and Metabolic and Echocardiographic Parameters**

<table>
<thead>
<tr>
<th></th>
<th>IGC Group (n = 24)</th>
<th>CGC Group (n = 23)</th>
<th>Control Group (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>123 ± 13*</td>
<td>122 ± 10</td>
<td>118 ± 11</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>5 ± 1</td>
<td>5 ± 4</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>19 ± 8*</td>
<td>16 ± 6</td>
<td>19 ± 12</td>
</tr>
<tr>
<td>GLU, mg/dl</td>
<td>206 ± 15*</td>
<td>125 ± 15†</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>CHOL, mg/dl</td>
<td>205 ± 13</td>
<td>204 ± 16</td>
<td>200 ± 14</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>37 ± 5</td>
<td>39 ± 6</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>TRIG, mg/dl</td>
<td>189 ± 27</td>
<td>182 ± 29</td>
<td>179 ± 32</td>
</tr>
<tr>
<td>Troponin, μg/l</td>
<td>16.3 ± 2.4*</td>
<td>3.1 ± 1.4†</td>
<td>9 ± 1.6</td>
</tr>
<tr>
<td>IS, %</td>
<td>39 ± 2*</td>
<td>33 ± 3†</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>EF, %</td>
<td>36 ± 3*</td>
<td>40 ± 4†</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>WMs</td>
<td>2.4 ± 0.4*</td>
<td>2.0 ± 0.3†</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>MPI</td>
<td>0.54 ± 0.14*</td>
<td>0.46 ± 0.09†</td>
<td>0.43 ± 0.15</td>
</tr>
</tbody>
</table>

Data are mean ± SD. *p < 0.05 versus control group; †p < 0.05 versus conventional glycemic control (CGC).

CHOL = plasma cholesterol; DBP = diastolic blood pressure; EF = ejection fraction; GLU = plasma glucose; HDL = high-density lipoprotein; HR = heart rate; IGC = intensive glycemic control; IS = infarct segment length; MPI = myocardial performance index; SBP = systolic blood pressure; TRIG = plasma triglycerides; WMs = wall motion score index.
Both immunohistochemistry and ELISA revealed markedly higher staining and levels of TNF-α and NFκB activation, as reflected by the selective analysis of the activated form of both p50 and p65, in all hyperglycemic ischemic specimens compared with normoglycemic specimens (p < 0.001). In hyperglycemic patients, staining and levels of TNF-α and both p50 and p65 were significantly more abundant in peri-infarcted area from the CGC group than peri-infarcted area from the IGC group (p < 0.001) (Fig. 3). Moreover, TNF-α levels in ischemic specimens were strongly dependent on glycemic control, as also reflected by the statistically significant correlation between mean plasma glucose levels during the treatment period and TNF-α concentrations (R = 0.41, p < 0.01). Finally, we have not shown any correlation between the amount of insulin administered (IU/kg) as well as the amount of insulin infused and the reduction of cardiac TNF-α levels (R = 0.1, p < 0.1; R = 0.03, p < 0.4).

TIGHT GLYCEMIC CONTROL REDUCES APOPTOTIC CELL DEATH IN THE ISCHEMIC HEART. Apoptotic cardiomyocytes were detected in all ischemic specimens from hyperglycemic patients, whereas the specimens from normoglycemic patients were totally free from apoptotic cell death, as evidenced by the absence of caspase-3–positive myocytes and tunel-positive myocytes. In hyperglycemic patients, immunostaining of caspase-3 was significantly higher in specimens from CGC patients compared with IGC group (Fig. 4). Tissue homogenates from heart specimens of both hyperglycemic groups showed a positive presence of caspase-3 activity that was
significantly greater in CGC patients compared with IGC patients ($p < 0.05$). Caspase-3 levels in the peri-infarct area significantly correlated with mean plasma glucose levels during the treatment period ($R = 0.43$, $p < 0.01$). Finally, we have not shown any correlation between the amount of insulin administered (IU/kg) as well as the amount of insulin infused and the reduction of cardiac caspase-3 levels ($R = 0.05$, $p < 0.3$; $R = 0.02$, $p < 0.1$).

**Discussion**

The most important message from this investigation of a different goal for glucose control in patients with hyperglycemia and AMI is that tight glycemic control during the ischemic insult has been associated with reduction of early post-infarction remodeling. Still, hyperglycemia has remained one of the most important prognostic predictors of...
early outcome in myocardial ischemia: stress hyperglycemia, during first AMI, amplifies oxidative stress, inflammatory immune reaction, and myocyte death, and worsens functional cardiac outcome. In our study, hyperglycemia was associated with higher troponin I levels and larger infarct size as well as myocardial TNF-α, NFκB-activated, caspase-3, and nitrotyrosine levels compared with normoglycemic patients. The present results seem to confirm our previous findings (6), because hyperglycemia was positively correlated with both infarct segment length and wall motion score index increases. Moreover, the increase in the MPI, which measures both systolic and diastolic parameters of ventricular function (14), indicates a worse functional outcome after AMI in hyperglycemic patients. Furthermore, the diminished diastolic filling time, the prolongation of mitral regurgitation, and the diminished effective ET in the hyperglycemic patients suggest that hyperglycemia might influence cardiac synchronization during AMI and, therefore, early post-infarction remodeling. Accordingly, several studies have shown that hyperglycemia is associated with adverse outcomes during AMI: elevated serum glucose levels in AMI patients, regardless of a known history of diabetes, was a risk factor for reinfarction, congestive heart failure, and future cardiovascular events (4). Although many aspects of the relation between glucose control and adverse outcome in hyperglycemic patients with AMI have not been
adequately defined, more extensive myocardial damage might be linked to a greater oxidative stress (15) as well as inflammatory process and activated T-cells that in turn evoke myocyte death. In this context, our data provide the evidence that hyperglycemia is associated with increased iNOS expression, $O_2^-$ production, and nitrotyrosine levels in the peri-infarcted area. Furthermore, increases in nitrotyrosine levels and $O_2^-$ production in the hyperglycemic myocardium are associated with increased myocardial injury, suggesting that increased nitric oxide production from iNOS enhances peroxynitrite formation, contributing to myocardial damage in hyperglycemic heart during myocardial infarction. Against this background is the observation that, in isolated rat hearts, acute exposure to high glucose increases iNOS gene expression, paralleled by a simultaneous increase of both nitric oxide and $O_2^-$ production (7). The interaction of $O_2^-$ with nitric oxide is very rapid and leads to inactivation of nitric oxide and production of the potent oxidant peroxynitrite (7). Because nitrotyrosine is considered a good marker of peroxynitrite formation (16), detection of high levels of nitrotyrosine in hyperglycemic hearts but not in normoglycemic hearts is strongly suggestive of increased generation of peroxynitrite. The observation that the increased apoptosis of myocytes, endothelial cells, and fibroblasts in heart biopsies from patients with diabetes (17) as well as in hearts from streptozotocin-induced diabetic rats (18) is selectively associated with levels of nitrotyrosine found in those cells supports our hypothesis. The mechanisms by which hyperglycemia increases apoptotic myocardial cell death remain to be explained, at least in part. The recent demonstration that cytokine-induced apoptosis is mediated by iNOS induction and peroxynitrite formation in primary cultures of neonatal rat myocytes (19) suggests a central role for pro-inflammatory cytokines in myocardial damage during ischemia, either acting directly on myocardial cells (20) or stimulating iNOS expression (21). Several inflammatory markers have been associated with cardiovascular events, including cytokines and growth factors, which are released by activated macrophages that, together with T-cells, are major cellular components (5). Moreover, we saw evidence that myocardial lymphocytes from patients with AMI are activated and that the clinical outcome is related to the intensity of the immunological activation. Both myocardial macrophages and T-lymphocytes from hyperglycemic patients with AMI had higher expression of HLA-DR (Dako) antigens compared with normoglycemic patients, suggesting that stress hyperglycemia is associated with enhanced T-cell activation. Lymphocyte activation seems to be necessary for the extension of the inflammatory process during AMI (5). Therefore, we found that hyperglycemic patients had higher myocardial levels of TNF-$\alpha$ and NFkB activation compared with normoglycemic patients. It might be speculated—following this line of thought—that during periods of ischemia, in addition to alterations in myocardial metabolism such as fatty

![Figure 4](image-url)
acids accumulation and insulin resistance, hyperglycemic myocardium might also incur further ischemic damage because of augmentation of the inflammatory response and increased production of superoxide radicals, which leads to increased apoptosis and consequently further damage in peri-infarcted area leading to impaired cardiac function.

The evidence that a more substantial drop in glucose in the first 24 h after AMI is associated with improved 30- and 180-day survival reaffirms that higher glucose levels are predictive of adverse outcome in AMI patients (4). Accordingly, our data evidenced that tight glycemic control in the immediate post-infarcted period, for almost 3 days, was associated with significantly less need for inotropic support and lower incidence of atrial fibrillation and pneumonia. This study also demonstrated that the reduction in glucose levels in the first period after AMI might improve cardiac function. After adjusting for baseline glucose and other clinical predictors, we found that, for every 10-mg/dl drop in glucose level between baseline and 6 days, there was an 11% relative decrease in the infarct segment length, 13% in the troponin levels, 11% in the wall motion scores, and a 12% decrease in the MPI as well as an 11% relative increase in the ejection fraction in hyperglycemic patients. The relation between the 6-day changes in glucose and cardiac function seemed to be independent of baseline glucose when both parameters were analyzed as continuous measures (p value for interaction = 0.57 at 6 days); however, this relation could not be reliably interpreted for patients with baseline glucose <126 mg/dl, because of the small glycemic changes in this group. In accordance with these data, the HI-5 (Hyperglycemia Intensive Insulin Infusion in Infarction) study (22) evidenced that, although insulin infusion therapy did not reduce the primary end point of mortality, it did reduce the secondary end points of heart failure (12.7%) compared with control subjects (22.8%; p = 0.04) as well as reinfarction at 3 months (2.4% vs. 6.1%; p = 0.05). Thus, although the investigators did not observe a significant reduction in overall mortality in patients receiving the insulin infusion, they suggested that “it remains possible that tight glycemic control with insulin therapy after acute myocardial infarction improves outcomes.” Moreover, the original DIGAMI (Diabetes Mellitus Insulin–Glucose Infusion in Acute Myocardial Infarction) study was the only randomized trial of glucose control in AMI to date to have achieved a significantly lower glucose level in the intervention arm compared with the control arm; it also happens to be the only randomized trial to have demonstrated a survival benefit associated with better glucose control (3). Nonetheless, all of these studies did not provide any evidence about the specific pathway transducing strict glycemic control in reduced post-infarction myocardial remodeling in hyperglycemic patients. As for mechanisms behind this association, a reduction of both inflammatory status and oxidative stress as well as a reduction of apoptotic death in the peri-infarcted area of IGC group might be responsible for less cardiac damage. According to this construct, our data evidenced that tight glycemic control (128 ± 15 mg/dl) in the immediate post-infarcted period, for almost 3 days, was associated with significant reduction of inflammatory cytokines, NFκB activation, oxidative stress, and apoptotic cell death. Because oxidative stress has been shown to induce inflammation through NFκB activation (23,24), we speculated that tight glycemic control by reducing oxidative stress might inhibit the cytokine expression and apoptosis in peri-infarcted area through NFκB inhibition. The limitations of this study should be noted. We cannot differentiate between the direct effects of endogenous or infused insulin and the effects of preventing hyperglycemia on cardiac changes of apoptosis and inflammation, because both occurred concomitantly in our study. In this context, the anti-inflammatory effects evoked directly by insulin through the suppression of cytokine production or signaling (25), favorable effects on coagulation and fibrinolysis (26), the antiapoptotic effect of insulin in experimental AMI independently of glucose (27) and on macrophage function (28) partially mediated by the prevention of hyperglycemia, might also have occurred. To minimize bias, we evaluated the correlation between the total amount of insulin administered and cardiac changes, and no overall correlation was observed. However, it should be noted that the anti-apoptotic and anti-inflammatory effects of insulin are attenuated in the presence of insulin resistance (29); moreover, evidence suggests that myocardial ischemia especially in the presence of hyperglycemia is associated with an important insulin resistance (30). Therefore, it is conceivable that the beneficial effects of insulin during myocardial infarction in the presence of hyperglycemia are attenuated. Accordingly, the epidemiological analysis from the DIGAMI 2 study (31) together with information from the study on patients in intensive care by van den Berghe (32) strongly support the concept that a meticulous glucose control rather than insulin treatment or the insulin dose (33) might be an important factor to improve cardiac outcome in hyperglycemic patients. In contrast, Díaz et al. (34), in a re-analysis of the CREATE-ECLA (Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation–Estudios Clínicos Latino America) study led to a separation of the effect of glucose-insulin-potassium therapy in the first 3 days and the next 27 days. Greater levels of mortality and congestive cardiac failure were observed in the first 3 days, whereas these indexes were significantly reduced after glucose-insulin-potassium therapy. The phase during which glucose-insulin-potassium-induced hyperglycemia occurred, mortality and heart failure increased, whereas both decreased significantly after the hyperglycemia receded and the potential benefit of insulin was exposed.

Conclusions

The present study demonstrates an additional aspect of how tight glycemic control might contribute to improved cardiac
outcome and reduce myocardial remodeling in hyperglycemic AMI patients.

Reprint requests and correspondence: Dr. Raffaele Marfella, Piazza Miraglia 2, 80138 Napoli, Italy. E-mail: raffaele.marfella@unina2.it.

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Key Words: apoptosis • glycemic control • inflammation • myocardial infarction • remodeling.