Valvular Heart Disease

Glucose-Insulin-Potassium Reduces the Incidence of Low Cardiac Output Episodes After Aortic Valve Replacement for Aortic Stenosis in Patients With Left Ventricular Hypertrophy

Results From the Hypertrophy, Insulin, Glucose, and Electrolytes (HINGE) Trial

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Background—Patients undergoing aortic valve replacement for critical aortic stenosis often have significant left ventricular hypertrophy. Left ventricular hypertrophy has been identified as an independent predictor of poor outcome after aortic valve replacement as a result of a combination of maladaptive myocardial changes and inadequate myocardial protection at the time of surgery. Glucose-insulin-potassium (GIK) is a potentially useful adjunct to myocardial protection. This study was designed to evaluate the effects of GIK infusion in patients undergoing aortic valve replacement surgery.

Methods and Results—Patients undergoing aortic valve replacement for aortic stenosis with evidence of left ventricular hypertrophy were randomly assigned to GIK or placebo. The trial was double-blind and conducted at a single center. The primary outcome was the incidence of low cardiac output syndrome. Left ventricular biopsies were analyzed to assess changes in 5′ adenosine monophosphate–activated protein kinase (AMPK), Akt phosphorylation, and protein O-linked β-N-acetylglucosaminidation (O-GlcNAcylation). Over a 4-year period, 217 patients were randomized (107 control, 110 GIK). GIK treatment was associated with a significant reduction in the incidence of low cardiac output state (odds ratio, 0.22; 95% confidence interval, 0.10 to 0.47; \( P = 0.0001 \)) and a significant reduction in inotrope use 6 to 12 hours postoperatively (odds ratio, 0.30; 95% confidence interval, 0.15 to 0.60; \( P = 0.0007 \)). These changes were associated with a substantial increase in AMPK and Akt phosphorylation and a significant increase in the O-GlcNAcylation of selected protein bands.

Conclusions—Perioperative treatment with GIK was associated with a significant reduction in the incidence of low cardiac output state and the need for inotropic support. This benefit was associated with increased signaling protein phosphorylation and O-GlcNAcylation. Multicenter studies and late follow-up will determine whether routine use of GIK improves patient prognosis.


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Key Words: heart valves ■ glucose ■ hypertrophy ■ insulin
The natural history of aortic stenosis is characterized by a protracted latent period accompanied by the development of left ventricular hypertrophy (LVH) as a compensatory mechanism to reduce wall stress. Although the operative mortality for aortic valve replacement (AVR) in the United Kingdom has remained ~3% despite an increasing elderly population with a greater risk profile, the presence of LVH is associated with increased risk and severity of postischemic myocardial injury and contractile dysfunction after AVR. LVH is also associated with an increased risk of post-surgical complications such as respiratory failure, renal failure, heart failure, and arrhythmias, and it has been identified as an independent predictor of in-hospital mortality after AVR.

The increased risk associated with LVH is attributable, at least in part, to inadequate myocardial protection including: from a combination of inadequate delivery of cardioplegia through a less dense capillary bed, increased diffusion distance for oxygen/metabolites from capillary to myocytes, impaired myocardial metabolism characterized by a shift away from free fatty acid use, uncoupling of glycolysis and glucose oxidation, and an increase in expression of mitochondrial uncoupling proteins, all resulting in a reduction in the ratio of phosphocreatine to ATP. The resultant early postischemic left ventricular dysfunction may not only prejudice late reverse remodelling of LVH after AVR and reduce the prognostic benefits of surgery, but may also predict the potentially important role of metabolic interventions.

Although the cardioprotective properties of glucose-insulin-potassium (GIK) remain controversial, in part because of the different contexts, doses, timing, and protocol of GIK used in different studies, there is a growing consensus supporting the utility of GIK in cardiac surgery. Multiple complementary mechanisms may contribute to GIK-related myocardial protection: (1) suppression of lipolysis, resulting in a reduction of circulating levels of free fatty acids available for cardiac metabolism; (2) an increase in insulin-related glucose flux and myocardial oxygen efficiency, increasing cardiac energy provision (ie, glycolytic ATP), which may augment myocardial glycogen levels and may mitigate the consequences of low myocardial pH; (3) beneficial augmentation of tricarboxylic acid intermediates (anaplerosis); and (4) through the pleiotropic signaling properties of insulin, including its antiapoptotic properties acting via phosphatidylinositol 3-kinase (PI3K), it may directly reduce myocardial injury. Activation of a cascade of what have been called reperfusion injury salvage kinases such as PI3K/protein kinase B (Akt) and AMP-activated protein kinase (AMPK), which have an established role in insulin signaling, may also have a role in cardioprotection. In addition to these established posttranslational modifications (eg, phosphorylation), insulin may promote modification of serine and threonine residues in proteins by O-linked β-N-acetylglucosamine (O-GlcNAc). However, the pertinence and contribution of these mechanisms in clinical context, especially in human LVH, remain speculative and unclear.

Although the results of AVR continue to improve, some patients exhibit a low cardiac output episode (LCOE) characterized by a period of left ventricular dysfunction requiring inotropic support. In some patients, this dysfunction is severe and prolonged, and the morbidity and mortality associated with LCOE continues to be substantial. In patients undergoing AVR with LVH, LCOE has been identified as the most common cause of death. In addition to increasing resource allocation, in the long term, myocardial injury sustained during surgery may result in heart failure. The need to improve myocardial protection in patients with LVH is well recognized and may reduce mortality, morbidity, and the resource implications of heart surgery. We report here a single-center, prospective, double-blind, randomized controlled trial to investigate the effect of GIK on the incidence of LCOE in patients with aortic stenosis and LVH undergoing AVR, either in isolation or in combination with coronary artery bypass grafting (CABG). To test the hypothesis that any cardioprotection delivered by GIK is contributed to by rapid and diverse posttranslational modifications, we assessed any changes in the established insulin-related signaling molecules Akt and AMPK and any increase in protein O-linked β-N-acetylglucosaminilation (O-GlcNAcylilation) in left ventricular biopsies from GIK-treated patients compared with control subjects.

Methods
An expanded Methods section appears in the online-only Data Supplement.

Study Design
We performed a prospective, single-center, double-blind, randomized, placebo-controlled trial of GIK in patients undergoing isolated AVR plus CABG with echocardiographic evidence of LVH as defined by a left ventricular mass index >134 g/m² for men or 100 g/m² for women. The study was approved by the South Birmingham Research Ethics Committee (reference 04/Q2707/23) and the Hospital Trust Board of Research. The trial was registered with the International Standardized Randomized Controlled Trial Number Register (reference ISRCTN 05758301). Patients were enrolled between October 2004 and June 2008. All research was performed in accordance with the Declaration of Helsinki within a research governance framework.

Surgery, Anesthesia, Cardiopulmonary Bypass, and Myocardial Protection
Anesthesia, cardiopulmonary bypass, and myocardial protection with intermittent antegrade cardioplegia using St Thomas solution buffered in cold blood were all standardized as previously described, except that aprotinin (Bayer) was used in all patients according to the departmental blood conservation strategy during this time, phenylepherine was used as the first-line vasoconstrictor, and patients were cooled to 32°C on bypass. Surgical technique was also standardized. In patients undergoing concomitant revascularization, distal anastomoses of all free grafts were performed first. Proximal graft anastomoses were performed during a period of partial aortic clamping. If the ascending aorta was dilated, it was replaced with a Gelweave interposition graft (Vascutek, Terumo, Renfrewshire, Scotland).

End Points
The primary outcome was the incidence of LCOE. This was defined a priori as a cardiac index of <2.2 L·min⁻¹·m⁻² refractory to appropriate intravascular volume expansion after correction or at-
tempted correction of any dysrhythmias.\textsuperscript{30} In the case of inotropic support being instituted as a result of an LCOE, a blinded end-points committee assessed all data. To qualify as an LCOE, a unanimous verdict was sought, but in the event of disagreement, contentious cases were discussed and a consensus opinion was reached. Secondary end points included comparison of cardiac index and the use of inotropes and vasoconstrictors. Perioperative myocardial infarction, assessed by an independent blinded cardiologist, was defined by the presence of new Q waves a minimum of 2 contiguous leads by postoperative day 4.

Statistical Analysis
All prespecified analyses were conducted according to the intention-to-treat principle. The study had a statistical power of 80\% to identify a relative risk of 0.50, which was statistically significant given an incidence of LCOE in the control group of 0.37 and a conventional 1-sided $\alpha$ of 0.025. Analyses were conducted with SAS software (version 9.1, SAS Institute, Inc, Cary, NC). $P$ values other than for the primary end point were nominal. Dichotomous outcomes were analyzed with the use of nonlinear mixed models, which included CABG as a patient-level covariate and surgeons as random effects. Continuous data were analyzed with the use of mixed models, which included CABG as a patient-level covariate and surgeons as random effects.\textsuperscript{31}

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree the manuscript as written.

Results

Study Population
A total of 220 patients were eligible for randomization; however, a pulmonary artery flotation catheter could not be placed in 3 patients, so they were excluded from the study. In total, 107 patients were randomized to receive the control infusion and 110 were randomized to receive the GIK infusion (Figure 1). Baseline preoperative characteristics were similar between the groups (Table 1). There were no

### Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>GIK (n=110)</th>
<th>Control (n=107)</th>
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<tbody>
<tr>
<td>Age, median (IQR), y</td>
<td>71.2 (63.4–76.5)</td>
<td>69.9 (65.0–74.4)</td>
</tr>
<tr>
<td>Female gender, n (%)</td>
<td>43 (39.1)</td>
<td>30 (27.5)</td>
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<tr>
<td>CCS class, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>I</td>
<td>46 (41.8)</td>
<td>42 (39.3)</td>
</tr>
<tr>
<td>II</td>
<td>50 (45.5)</td>
<td>53 (49.5)</td>
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<tr>
<td>III</td>
<td>13 (11.8)</td>
<td>12 (11.2)</td>
</tr>
<tr>
<td>IV</td>
<td>1 (0.9)</td>
<td>0</td>
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<tr>
<td>NYHA class, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>I</td>
<td>38 (34.5)</td>
<td>37 (34.6)</td>
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<tr>
<td>II</td>
<td>16 (14.5)</td>
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</tr>
<tr>
<td>III</td>
<td>51 (46.4)</td>
<td>51 (47.7)</td>
</tr>
<tr>
<td>IV</td>
<td>5 (4.5)</td>
<td>4 (3.7)</td>
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<tr>
<td>Previous MI, n (%)</td>
<td>5 (4.5)</td>
<td>3 (2.8)</td>
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<tr>
<td>Priority, n (%)</td>
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<tr>
<td>Elective</td>
<td>99 (90)</td>
<td>95 (88.8)</td>
</tr>
<tr>
<td>Urgent</td>
<td>11 (10)</td>
<td>12 (11.2)</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
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<tr>
<td>Current</td>
<td>3 (2.7)</td>
<td>2 (1.9)</td>
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<tr>
<td>Previous</td>
<td>25 (22.7)</td>
<td>36 (33.6)</td>
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<tr>
<td>Never</td>
<td>82 (74.5)</td>
<td>69 (64.5)</td>
</tr>
<tr>
<td>PVD, n (%)</td>
<td>2 (1.8)</td>
<td>2 (1.83)</td>
</tr>
<tr>
<td>TIA, n (%)</td>
<td>2 (1.8)</td>
<td>8 (7.3)</td>
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<tr>
<td>CVA, n (%)</td>
<td>2 (1.8)</td>
<td>2 (1.83)</td>
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<tr>
<td>Previous CEA, n (%)</td>
<td>1 (0.9)</td>
<td>2 (1.83)</td>
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<tr>
<td>Coronary artery disease, n (%)</td>
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<tr>
<td>None</td>
<td>82 (74.5)</td>
<td>79 (73.8)</td>
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<tr>
<td>Single vessel</td>
<td>11 (10)</td>
<td>8 (7.5)</td>
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<tr>
<td>Double vessel</td>
<td>12 (10.9)</td>
<td>13 (12.1)</td>
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<tr>
<td>Triple vessel</td>
<td>5 (4.5)</td>
<td>7 (6.5)</td>
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<tr>
<td>EuroSCORE median, IQR</td>
<td>6 (4–7)</td>
<td>6 (4–7)</td>
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<tr>
<td>Logistic EuroSCORE median, IQR</td>
<td>4.4 (2.7–7.1)</td>
<td>4.5 (2.8–7.7)</td>
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CCS indicates Canadian Cardiovascular Society; NYHA, New York Heart Association; MI, myocardial infarction; PVD, peripheral vascular disease; TIA, transient ischemic episode; CVA, cerebrovascular accident; CEA, carotid endarterectomy; A/A, aortic valve area; EF, ejection fraction; IVSD, interventricular septum in diastole; LVEDD, left ventricular end-diastolic diameter; and PWDD, posterior wall dimensions in diastole.
differences in demographics and risk profiles between the groups, with a median European System for Cardiac Operative Risk Evaluation (EuroSCORE) in the GIK arm of 4.4 (interquartile range [IQR], 2.7 to 7.1) and in the control arm of 4.5 (IQR, 2.9 to 7.7). We found that 191 patients had lone aortic stenosis and 29 had mixed aortic valve disease, of which the predominant lesion was aortic stenosis. All patients had preoperative transthoracic echocardiography (Table 1).

There were 146 patients scheduled for isolated AVR and 75 patients scheduled for concomitant CABG. Two of the 75 patients scheduled for additional CABG did not receive this additional procedure because of small coronary arteries. Seven patients underwent additional procedures. One patient underwent mitral valve annuloplasty. One patient was found to have a structurally normal and noncalcified aortic valve, and the outflow tract obstruction was due to an undiagnosed subaortic membrane, which was resected. Five patients underwent ascending aortic replacement as a result of postsystolic dilatation. Median bypass and cross-clamp times were similar in both groups, as was the number of bypass grafts required. Valve choice was at the discretion of the surgeon; a wide variety of prostheses were used.

GIK administration is associated with hyperglycemia; therefore, all patients in this trial were closely monitored with hourly blood glucose measurements throughout the study period. Patients with hyperglycemia were treated aggressively with supplemental insulin aiming for a blood glucose <10 mmol/L. Despite this approach, patients randomized to GIK demonstrated a rise in blood glucose that was maintained throughout the infusion period (mean reperfusion glucose, 11.8 mmol/L for GIK-treated patients versus 7.3 mmol/L for control subjects; P=0.01). Once the period of GIK infusion had ended, blood glucose levels were similar between the groups (mean glucose, 7.3 and 7.7 mmol/L for the GIK and control groups, respectively; P=0.78; Figure 2). GIK infusion was accompanied by a similar rise in insulin levels to >4 times baseline (mean reperfusion, 478 and 66 μU/mL for the GIK and control groups; P=0.0001), which returned to baseline after discontinuation of GIK therapy. The high level of insulin associated with GIK also led to suppression of plasma free fatty acids throughout ischemia and the 6-hour period of reperfusion during which GIK was infused (mean reperfusion, 483 and 892 μU/mL for the GIK and control groups; P=0.0001).

**Primary Outcome**

An LCOE was diagnosed in 47 patients: 11 of 110 (10.0%) in the GIK group and 36 of 107 (33.6%) in the control group. The use of GIK was associated with a significant reduction in the incidence of low cardiac output state (odds ratio, 0.22; 95% confidence interval, 0.10 to 0.47; P=0.0001; Table 1). Prespecified subgroup analyses revealed no heterogeneity in the effect of GIK on LCOE (Figure 3).

**Secondary End Points**

**Hemodynamic Data**

Cardiac index was higher in the GIK group from the beginning of treatment until 12 hours after removal of the cross-clamp (P=0.0001; Figure 2). Throughout the study, heart rate, central venous pressure, and pulmonary artery wedge pressure were similar between groups.

**Inotrope and Vasoconstrictor Use**

The use of GIK was associated with a significant reduction in the use of inotropes during the infusion period (n=35 patients [33.0%] in the control group versus 13 patients [11.9%] in the treatment group; odds ratio, 0.27; 95% confidence interval, 0.13 to 0.57; P=0.0006). This difference continued from 6 to 12 hours (Table 2). The use of a GIK infusion was also associated with an increase in the prevalence of vasoconstrictor use in the period between baseline and 6 hours after removal of the aortic cross-clamp (48 patients [45.3%] in the control group versus 70 patients [64.2%] in the GIK group; P=0.005). The difference in vasoconstrictor use was reduced in the 6- to 12-hour period, but evidence of increased vasoconstrictor requirements remained (P=0.02; Table 2).

**Myocardial Injury**

ECG evidence of myocardial injury occurred in 9 patients (8%) in the control group and 6 patients (5.5%) in the GIK group (P=0.67). When accounting for both surgeon and

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**Figure 2.** Mean cardiac index (L/m²) and blood glucose levels (mmol/L) between treatment groups during the period of hemodynamic monitoring. Error bars represent 95% confidence interval (CI) for the mean (P<0.0001).

**Figure 3.** Effect of GIK on LCOE in predefined subgroups.
intention to perform CABG using a continuous outcome, we found no significant difference in plasma troponin levels between treatment groups. At 6 hours, the difference in means was −0.01 (95% confidence interval, −0.09 to 0.07; \( P=0.81 \)).

**Akt and AMPK Signaling and Protein O-GlcNAcylation**

Akt, AMPK, and O-GlcNAcylation was studied in 16 patients (8 GIK and 8 control subjects) undergoing isolated AVR. There were no significant differences in the preoperative demographics or in the echocardiographic markers of function between groups. Immunoblotting demonstrated a \( \approx 2.5 \)-fold increase in the ratio of phospho-Akt to pan-Akt (\( P=0.03 \)) and a \( \approx 1.7 \)-fold increase in the ratio of phospho-AMPK to AMPK (\( P=0.0004 \); Figure 4A and B, respectively). Visual inspection suggested that there was more prominent O-GlcNAcylation in the GIK group. Individual band analysis suggested that in the GIK group a band of 60 kDa manifested \( \approx 1.85 \)-fold more O-GlcNAcylation than control subjects (\( P=0.004 \)). Protein loading was assessed by GADPH and β-tubulin. Band densitometry was performed with the Quantity One package.

**Discussion**

In this trial of GIK in addition to standard myocardial protection during AVR and combined AVR and CABG, GIK treatment resulted in a significant reduction in LCOE. There was an associated significant reduction in the use of inotropic support, but there was an increase in the need for vasoconstrictor therapy. The addition of GIK, however, had no effect on the incidence of postoperative myocardial infarction on ECG criteria or on serial plasma troponin release. We also report that GIK therapy was associated with an increase in dynamic posttranslational protein modification, including AMPK and Akt phosphorylation and protein O-GlcNAcylation, that may have contributed to the beneficial cardioprotective effect of GIK.

The results of this trial are likely to correspond to real-world clinical practice; the median logistic EuroSCORE of the patients not recruited during the study period was 5.1 (IQR, 2.3 to 8.2) compared with 4.5 (IQR, 2.8 to 7.1) for patients enrolled, suggesting a nonlikelihood of bias. Additionally, the incidence of inotropic use in patients randomized to control is similar to that of patients not enrolled (data not shown).

We previously studied the use of GIK in patients undergoing isolated CABG and demonstrated a similar improvement in cardiac hemodynamic performance and a reduction in the need for inotropic support.\(^1\) However, the magnitude of effect seen in relation to GIK therapy in this study was significantly greater than that which we have previously reported. All the patients randomized in this trial manifested significant LVH secondary to aortic stenosis. LVH has been shown to be associated with an impairment in cardiac energetics manifested by a reduction in the ratio of phosphocreatine to ATP on magnetic resonance spectroscopy and a downregulation in metabolism.\(^10,32\) This energetically impaired myocardium may be more vulnerable to ischemia/reperfusion injury and may thus derive greater benefit from improved metabolism.

In both of these studies, although hemodynamic indexes were improved, no significant effect on plasma troponin T (TnT) levels was demonstrated. The discrepancy between improved hemodynamic performance and TnT release has led to speculation that rather than improving myocardial protection, GIK increases cardiac output simply by vasodilatation.\(^33\) Consistent with this hypothesis, we have detected an increase in the need for vasoconstriction in patients treated with GIK. Although there is evidence to suggest that TnT release has modest prognostic value in cardiac surgery,\(^34\) myocardial protection in cardiac surgery is highly efficacious, and the majority of patients undergoing surgery have only a modest TnT rise of insignificant value. Myocardial necrosis with TnT release is the end stage of a series of reversible and finally irreversible steps that the myocytes undergo during ischemia/reperfusion, and significant TnT release occurs in only a minority of patients undergoing cardiac surgery. Myocardial stunning, however, is more common after cardiac surgery, is likely to significantly contribute to LCOE, is not necessarily associated with TnT release,\(^35\) and may be responsive to GIK therapy. The dissociation of the influence of GIK on LCOE

<table>
<thead>
<tr>
<th>Table 2. Study Outcomes, GIK Group Compared With the Control Group</th>
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<td><strong>Outcome</strong></td>
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<td>-----------------------------------------</td>
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<tr>
<td>Primary outcome, n (%)</td>
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<tr>
<td>Low cardiac output syndrome</td>
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<tr>
<td>Secondary outcomes, n (%)</td>
</tr>
<tr>
<td>Prevalence of inotrope use in first 6 h*</td>
</tr>
<tr>
<td>Inotrope use, 6–12 h</td>
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<tr>
<td>Vasoconstrictor use in first 6 h*</td>
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<tr>
<td>Vasoconstrictor use, 6–12 h</td>
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<tr>
<td>Insulin use in first 6 h</td>
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<tr>
<td>Insulin use, 6–12 h</td>
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<tr>
<td>Continuous outcome, n (%)</td>
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<td>Cardiac index at 6 h, mean (SD), L · min⁻¹ · m⁻²</td>
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</table>

*Before cardiopulmonary bypass, at cardiopulmonary bypass, and at 0 to 6 hours.
†Difference in means (95% confidence interval).
and TnT release may thus be explained by the impact of GIK on myocardial stunning rather than necrosis per se.

Recovery from perioperative myocardial stunning involves normalization of intermediary and oxidative metabolism, resulting in restitution of myocardial energy reserves, reversal of cell swelling, and gradual accumulation of the total adenine nucleotide pool. The more established role for insulin in cardioprotection is through amelioration of myocardial metabolism, including a switch from deleterious free fatty acid metabolism to more efficient glucose metabolism. Such a role for GIK has been extensively established in experimental studies. However, in addition to the metabolic benefits conferred by GIK, recent studies have adduced a role for pH homeostasis and insulin-mediated antiapoptotic and cardioprotective signaling pathway activation. Thus, although it is likely that the main cardioprotective action of GIK is through a shift to more efficient metabolism, we investigated whether myocardial signaling events contributed to GIK-related myocardial protection.

Insulin, acting through the tyrosine kinase activity of the insulin receptor, phosphorylates and activates insulin receptor substrate-1 and -2, which in turn activate PI3K. PI3K activation generates phosphoinositide-3,4,5 triphosphate, which mediates the phosphorylation and activation of Akt. In cardiomyocytes, Akt has been shown to protect against apoptosis after ischemia/reperfusion injury, and as a corollary, pharmacological inhibition of Akt has been shown to abolish the cardioprotective effect of insulin. To the best of our knowledge, the present study is one of the first to confirm, in accordance with the extensive animal model literature associating Akt phosphorylation with insulin treatment, that GIK activates Akt, which is likely to contribute to the benefits noted here. We also demonstrate a significant increase in AMPK phosphorylation. AMPK activation is known to play a crucial role in the regulation of cardiac energy metabolism and is thought to be an adaptive mechanism in cardiac ischemia. However, the finding that AMPK phosphorylation is enhanced in the context of GIK may be considered paradoxical. This apparent “paradox” is predicated on the observation that insulin, via activation of Akt, inhibits AMPK by phosphorylation of Ser 485/491 of Akt. Reconciling these observations, it appears that although high insulin concentrations, especially in the absence of lipid, do indeed inhibit AMPK activity, the inhibitory effect of insulin on AMPK activity is relieved when insulin and lipid concentrations are more physiological (palmitate, 0.2 to 1.2 mmol/L). Accordingly, we speculate that GIK in this study was able to activate Akt with its attendant benefits but suppressed systemic lipid levels to such a permissive level that the beneficial effects of AMPK activation were manifest during ischemia/reperfusion. The magnitude of kinase activation in this study, however, may have been mitigated by hyperglycemia secondary to glucose infusion. There is a compelling association between hyperglycemia and increased mortality from myocardial ischemia, whether in the context of myocardial infarction or coronary surgery. This effect of hyperglycemia is due, at least in part, to the hyperglycemia-induced decrease in myocardial Akt activation and may have been relevant to the present GIK study.

We also observed an increase in protein O-GlcNAcylation. This is the first evidence of O-GlcNAcylation in humans and its potential interaction with insulin. Protein O-GlcNAcylation refers to the posttranslational modification of serine and threonine residues of nuclear and cytoplasmic proteins by O-GlcNAc. This modification is emerging as a key regulator of a number of critical biological processes, including nuclear transport, translation and transcription, signal transduction, and apoptosis, and in these settings, activation appears to be an endogenous stress response designed to enhance cell survival. In the isolated perfused heart, ischemia has been shown to increase overall O-GlcNAc levels, suggesting that this endogenous stress-activated pathway is active in the heart and that increased

![Graph](http://circ.ahajournals.org/)

**Figure 4.** Western blot analysis of AMPK, phospho-AMPK, Akt, and phospho-Akt of human left ventricular biopsy-derived protein in control (Ctrl) and GIK-treated samples. The results are expressed as (A) ratio of Akt to phospho-AKT and (B) ratio of AMPK to phospho-AMPK, each result having first been normalized against β-tubulin, the loading control (n=8 respectively). These blots show significantly increased GIK-mediated Akt and AMPK phosphorylation.
O-GlcNAc levels improved contractile function and decreased tissue injury after reperfusion.49,50 We speculate that insulin increases O-GlcNAcylation by increasing intracellular glucose flux and hence delivers cardioprotection. Which proteins are modified and the specificity of this process remain the subjects of intensive investigation.

**Conclusions**

In patients with significant LVH, the addition of perioperative GIK therapy to standard myocardial protective techniques resulted in a significant reduction in LCOE. We observed a significant reduction in inotrope use but a corresponding rise in vasoconstrictor use, secondary to the known vasodilatation associated with insulin, that consequently resulted in no reduction in postoperative resource use. Although the mortality in this trial was significantly lower than predicted, this study was not powered to detect a difference in mortality. Although we present novel human data supporting the role of metabolic modulation, insulin signaling pathway activation (eg, via traditional pathways such Akt and AMPK activation), and protein O-GlcNAcylation in insulin-mediated cardioprotection, these data are preliminary and necessarily only hypothesis generating. Further evidence is required to ascertain whether this protective pathway is responsible for improved myocardial protection in the context of GIK therapy.

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**Disclosures**

None.

**References**


**CLINICAL PERSPECTIVE**

Although advances in surgical and myocardial preservation techniques have substantially reduced the operative risks associated with cardiac surgery, aortic valve replacement surgery is increasingly being performed in elderly high-risk patients who are at high risk of hemodynamic insults such as low cardiac output syndrome. In this single-center, randomized, double-blind, placebo-controlled trial in patients undergoing aortic valve replacement, glucose-insulin-potassium improved the hemodynamic status of treated patients compared with those on placebo. The mechanisms contributing to this beneficial effect of glucose-insulin-potassium are complex and include metabolic enhancement. We demonstrate for the first time in humans that glucose-insulin-potassium augments myocardial signalling by increasing protein O-linked β-N-acetylglucosamination of select proteins and Akt/5′-AMP–activated protein kinase phosphorylation. Although requiring replication, this study provides a rationale for the instigation of glucose-insulin-potassium during cardiac surgery and mandates a more detailed assessment of the link between O-linked β-N-acetylglucosamination and cardioprotection during ischemia/reperfusion in humans.
Glucose-Insulin-Potassium Reduces the Incidence of Low Cardiac Output Episodes After Aortic Valve Replacement for Aortic Stenosis in Patients With Left Ventricular Hypertrophy: Results From the Hypertrophy, Insulin, Glucose, and Electrolytes (HINGE) Trial


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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Patients

Eligible patients were at least 18 years of age undergoing first time aortic valve replacement surgery (± CABG) for aortic stenosis with echocardiographic evidence of LVH as defined by a left ventricular mass index greater than 134 g.m\(^{-2}\) for men, or 100 g.m\(^{-2}\) for women\(^{1, 2}\). Patients hospitalised for emergency cardiac surgery, or surgery involving other heart valves, were excluded. Also excluded were pregnant women, and those with diabetes mellitus, renal failure requiring dialysis or atrial fibrillation.

Randomisation

The allocation of treatment or placebo is randomly determined in a blinded fashion and includes stratification need for concomitant CABG. The randomisation schedule for the allocation of treatment or placebo uses a blinded minimisation procedure designed by Professor Freemantle, Health Care Evaluation Group, University of Birmingham.

Treatment Groups

The treatment arm received a central GIK infusion consisting of 500ml of 40% glucose, 35IU Actrapid® insulin (Novo Nordisk A/S, Bagsvaerd, Denmark) 50mmol of potassium chloride (KCl)) run at 0.75ml.kg\(^{-1}\).h\(^{-1}\) rounded to the nearest 10ml.h\(^{-1}\) starting at sternotomy and finishing six hours following release of the AXC. The control group received 5% dextrose run at 0.75ml.kg\(^{-1}\).h\(^{-1}\) rounded to the nearest 10ml.h\(^{-1}\) starting at sternotomy and finishing six hours following release of the aortic cross clamp (AXC). To ensure blinding of investigators,
the solutions were prepared by independent operating department practitioners who wrapped the trial solution bags of placebo/GIK in opaque tape to conceal the identity of the solution and simply labelled as “trial solution”.

**Echocardiography**

All pre-operative echocardiograms were performed within one week of AVR. Left ventricular ejection fraction (LVEF), left ventricular dimensions, LVMI, and degree of AS were determined prior to surgery from M-mode, 2D and Doppler echocardiography (Table 1). Measurements were made according to the American Society of Echocardiography Guidelines and averaged from 3-5 cycles. Diastolic measurements were used to calculate LVMI by means of the method described by Devereaux. Ejection fraction was calculated using the Biplane Simpson’s method.

**Trial Solutions**

GIK/placebo therapy was administered from induction of anaesthetic until six hours following aortic cross clamp (AXC) removal at a rate of 0.75mlkg\(^{-1}\)hr\(^{-1}\) as previously described.

**Methods**

**Haemodynamic Investigations and Markers of Myocardial Injury**

Following induction of anaesthesia, a pulmonary artery flotation catheter was placed. The cardiac output was measured using a thermodilution technique and the recorded value was the mean of three measurements. Recorded values for pulmonary artery pressure, pulmonary artery wedge pressure (PAWP) and central venous pressure (CVP) were obtained at end-expiration from graphic recordings. Baseline haemodynamic studies were performed and repeated before and after administration of protamine and at 2,4,6,9 and 12
hours following cross-clamp removal. Serum cardiac Troponin T (cTnT) samples were collected at baseline, 6, 12 and 24 hours following AXC removal and analysed in batches with the ECLIA Troponin T assay (Roche Diagnostics, Burgess Hill, West Sussex, UK) using a Modular Analytics E170 using a commercial assay. Pre-operative and post-operative day 4 electrocardiograms (ECGs) were obtained off pacing if possible.

**Free fatty acid, glucose and insulin**

Serial samples were taken at baseline, at cross clamp removal, 6 and 12 hours following AXC removal. Plasma was analysed for free fatty acids by enzymatic colorimetry (WAKO Chemicals, Alpha Laboratories, Eastleigh, U.K.), for glucose with a Beckman Glucose analyser II (Beckman Instruments, Fullerton, USA) and insulin by an immunochemiluminometric assay (MolecularLight Technology, Cardiff, U.K.).

**Left Ventricular Biopsies and Western Blotting Protocol**

Full thickness LV biopsies were taken from the left ventricular free wall as previously described. These biopsies were taken prior to AXC, prior to removal of the AXC and after 10 minutes reperfusion. Biopsies were immediately snap-frozen in liquid nitrogen and stored at -80°C.

**Protein Extraction and Western Blotting**

Myocardial biopsies were homogenized using the Polytron-Aggregate system (Kinematica, Luzern, Switzerland), in extraction buffer (25 mM Tris•HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with 1x Complete protease inhibitor cocktail/10ml buffer (Roche, Basel, Switzerland) and 1x PhosSTOP phosphatase inhibitor cocktail/10ml (Roche, Basel, Switzerland) as well as Na-orthovanadate (1mM). To inhibit the action of liberated tissue proteases 1.67uM phenylmethanesulphonyl fluoride (PMSF) (protease inhibitor) was also included. For O-linked GlcNAc studies the extraction buffer was supplemented with 10μM O-(2-acetamido-2-deoxy-D-glucopyranosylidene) amino-N-
phenylcarbamate (PUGNAc) (Sigma-Aldrich) (O-GlcNAcase inhibitor). No PhosSTOP was used in the latter extraction protocol. Tissue suspensions were kept on ice during the extraction procedure. Protein concentrations were determined using the BCA protein assay (Thermo Scientific, Rockford, IL).

Proteins were electrophoretically separated on 4-12% graduated SDS-PAGE gels (Invitrogen, Carlsbad, CA) and transferred to PVDF membranes overnight. For O-GlcNAc studies, transfer took place over one hour at 400mA to nitrocellulose membrane (Amersham Life-Science). After blocking with 5% fat free milk (in the case of kinase blotting supplemented with 50mM NaF acting as a phosphatase inhibitor) or 3% BSA (Sigma-Aldrich), membranes were incubated with antibodies directed against phosphorylated AKT (ser 473) or total AKT (both Abcam, Cambridge, UK), and phosphorylated AMPK (Thr 172) or total AMPK (both Cell Signaling Technology, Danvers, MA) or O-GlcNAc - CTD110.6 (Covance, Emeryville, CA). After stripping, membranes were reprobed with antibodies against the total form of the respective kinases. GAPDH (Abcam, Cambridge, UK) or β-tubulin (Abcam, Cambridge, UK) was used as a loading control. The immunoreactive signals were detected by chemiluminescence (ECL Advance, Amersham, Bucks, UK) and quantified using the Quantity One software package (Bio-Rad Laboratories, Herts, UK).

SUPPLEMENTAL REFERENCES


3. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, Picard MH, Roman MJ, Seward J, Shanewise JS, Solomon SD, Spencer KT, Sutton MSJ, Stewart WJ. Recommendations for chamber quantification: a report from the American Society of
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