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Systemic glucose-insulin-potassium reduces skeletal muscle injury, kidney injury, and pain in a murine ischaemia-reperfusion model

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Aims

Glucose-insulin-potassium (GIK) is protective following cardiac myocyte ischaemia-reperfusion (IR) injury, however the role of GIK in protecting skeletal muscle from IR injury has not been evaluated. Given the similar mechanisms by which cardiac and skeletal muscle sustain an IR injury, we hypothesized that GIK would similarly protect skeletal muscle viability.

Methods

A total of 20 C57BL/6 male mice (10 control, 10 GIK) sustained a hindlimb IR injury using a 2.5-hour rubber band tourniquet. Immediately prior to tourniquet placement, a subcutaneous osmotic pump was placed which infused control mice with saline (0.9% sodium chloride) and treated mice with GIK (40% glucose, 50 U/l insulin, 80 mEq/L KCl, pH 4.5) at a rate of 16 µl/hr for 26.5 hours. At 24 hours following tourniquet removal, bilateral (tourniqueted and non-tourniqueted) gastrocnemius muscles were triphenyltetrazolium chloride (TTC)-stained to quantify percentage muscle viability. Bilateral peroneal muscles were used for gene expression analysis, serum creatinine and creatine kinase activity were measured, and a validated murine ethogram was used to quantify pain before euthanasia.

Results

GIK treatment resulted in a significant protection of skeletal muscle with increased viability (GIK 22.07% (SD 15.48%)) compared to saline control (control 3.14% (SD 3.29%)) ($p = 0.005$). Additionally, GIK led to a statistically significant reduction in gene expression markers of cell death (CASP3, $p < 0.001$) and inflammation (NOS2, $p < 0.001$; IGF1, $p = 0.007$; IL-1β, $p = 0.002$; TNFα, $p = 0.012$), and a significant reduction in serum creatine kinase ($p = 0.004$) and creatinine ($p < 0.001$). GIK led to a significant reduction in IR-related pain ($p = 0.030$).

Conclusion

Systemic GIK infusion during and after limb ischaemia protects murine skeletal muscle from cell death, kidneys from reperfusion metabolites, and reduces pain by reducing post-ischaemic inflammation.

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Keywords: Skeletal muscle, Ischaemia, Reperfusion, Glucose-insulin-potassium, Tourniquet

Article focus

- The impact of glucose-insulin-potassium (GIK) on the rate and nature of ischaemia-reperfusion (IR) injury in skeletal muscles in an animal model.

Key messages

- Systemic infusion of GIK protects murine skeletal muscle from cell death, kidneys from reperfusion metabolites,

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and decreases pain by reducing post-ischaemic inflammation.
- GIK may reduce local and systemic morbidity from all types of IR injury, including musculoskeletal or vascular trauma, elective surgery requiring tourniquet use, and even compartment syndrome.

Strengths and limitations

- The present study uses a small animal model. In the future, large animal models and human trials will be required to corroborate our findings.
- The present study focuses on markers of cell injury; effects of GIK on other systems have not been studied and require further elucidation.
- Our findings strongly support the need for additional in vitro and in vivo trials to further define the role of GIK in the treatment of musculoskeletal injuries which if corroborated, could result in a significant clinical benefit for patients.

Introduction

Limb injuries account for over 14 million emergency department visits per year and cost an estimated $80 billion,[1] with exsanguinating injuries being a key factor in fatalities both in civilian and military trauma.[2,3] Tourniquets are used as the primary preventative measure against limb exsanguination and to create a bloodless surgical field, although are not benign.[4] The injuries that result from tourniquet use occur with varying severity and can lead to acute skeletal muscle apoptosis and necrosis.[5] While ischaemia often causes the initial insult, reperfusion is what causes the most significant damage to the muscle in what has been termed an ischaemia-reperfusion (IR) injury. In addition to acute muscle injury, IR can also cause long-term sequelae, in worst-case scenarios leading to limb paralysis and amputation.[6]

Preventing IR injury has been an area of interest in cardiology after myocardial infarction, but has not gained traction in the orthopaedic realm. Specifically, glucose-insulin-potassium (GIK), a unique treatment for the prevention of myocardial IR injury following myocardial infarction or cardiac surgery, may have utility in both cardiac and skeletal muscle. Since first being tested in 1962,[7] several studies have shown that GIK may reduce myocardial IR injury by increasing glycogen and adenosine triphosphate (ATP)[8] and reducing free fatty acids (FFAs) and reactive oxygen species (ROS).[9] Subsequent clinical studies suggest that GIK reduces mortality following myocardial infarction and cardiac surgery.[10,11] Despite positive results in the cardiac literature, only one study has investigated the effect of GIK on skeletal muscle, and their focus was on glycogen depletion during fasting.[12] Thus, the potential beneficial effect of GIK on skeletal muscle has not been rigorously investigated.

Like cardiac muscle, skeletal muscle IR injury occurs through ischaemic ATP depletion and reperfusion-mediated ROS production.[13,14] ATP depletion destabilizes the electrochemical gradient of the sarcolemma, leading to increased intracellular proteolytic enzyme number and activity. Excess ROS increases sarcolemma permeability, further increasing intracellular proteolytic enzyme number and activity. These enzymes degrade the muscle cell, releasing large quantities of muscle breakdown products including myoglobin, creatinine kinase, and lactate dehydrogenase, which can lead to kidney failure and death.[13] Since GIK has been shown to mitigate these processes in cardiac muscle,[8,9,15] we strongly believe that GIK can decrease skeletal muscle IR injury.

Considering the support for GIK in the cardiac literature and the analogous cellular mechanisms through which both cardiac and skeletal muscle sustain an IR injury, we hypothesized that GIK could improve muscle viability following skeletal muscle IR injury. Using a prolonged tourniquet mouse model, the primary aim of this study was to evaluate the ability of GIK to reduce skeletal muscle injury, and the secondary aim was to evaluate its ability to reduce IR injury-related morbidity.

Methods

Experimental animals. Following approval by our institution's Committee on Animal Research, experiments were performed on a total of 20 (10 control, 10 GIK) 12-week-old C57BL/6 male mice (Mus musculus). As a pilot study evaluating the utility of GIK in reducing IR injury in skeletal muscle, single-sex, male mice were chosen to limit confounding due to sex-related variabilities. A priori sample size calculation determined that 20 mice would be required to detect a significant difference in muscle viability following prolonged tourniquet use (10 control, 10 GIK). There were no preset criteria for including or excluding animals during the experiments. Mice were purchased from an approved vendor (The Jackson Laboratory, USA) and maintained on a 12-hour light/dark cycle with food and water provided ad libitum. There was no protocol for randomization to control or treatment groups; the samples were sequentially used for control and treatment arm based on animal identification number allowing for quasi-randomization. The present study adheres to the ARRIVE guidelines (Supplementary Material).

GIK and control infusions. GIK was continuously infused at 16 µl/hr (40% glucose, 50 U/l insulin, 80 mEq/L KCL, pH 4.5) using a dorsally placed subcutaneous osmotic pump (Alzet, USA). The concentration of GIK was determined using a modification of the previously described Rackley formula.[16] Sodium chloride (0.9%) was continuously infused at 16 µl/hr using a dorsally placed subcutaneous osmotic pump as a control cohort. Subcutaneous osmotic infusion was chosen over intravenous infusion as it is rapid, inexpensive, and does not require continuous vascular access that tethers the animal to equipment.[17]

After induction of anaesthesia with isoflurane inhalation (1% to 5%), the dorsum of the mice was clipped and sterilely prepped with betadine. A 5 mm incision was made, the skin was bluntly dissected to create a subcutaneous pocket, and the osmotic pump was inserted.

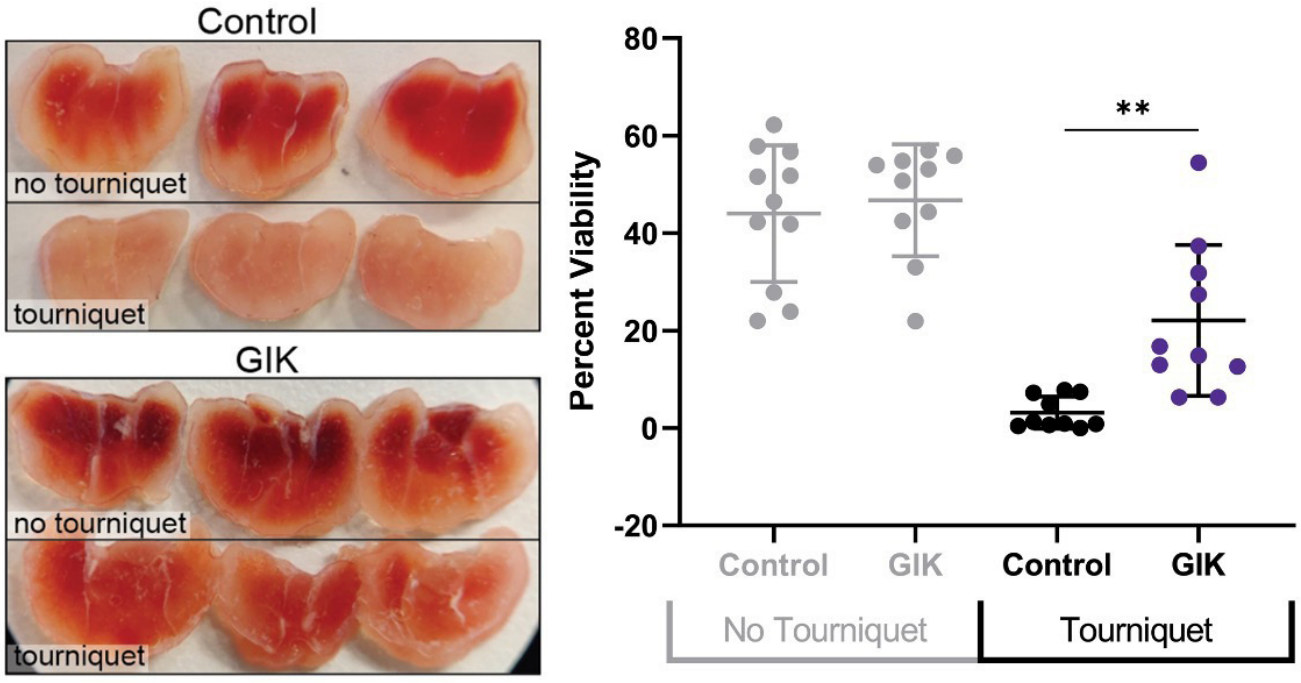

Fig. 1

Representative images of the gastrocnemius triphenyltetrazolium (TTC) stain and quantification of the gastrocnemius TTC stain demonstrating significantly greater TTC stain in the glucose-insulin-potassium (GIK) cohort compared to the control cohort tourniqueted limbs (22.1% (n = 10) vs 3.1% (n = 10), p = 0.006). **p ≤ 0.01.

Experimental units were randomly allocated to either control or GIK in an alternating fashion. 7-0 braided absorbable suture was used for skin closure.

Tourniquet procedure. After osmotic pump insertion, mice were subjected to a unilateral, proximal hindlimb tourniquet using a 4.5-ounce orthodontic rubber band placed via McGivney Hemorrhoidal Ligator (Miltex, Germany).[5] Tourniquets remained in place for 2.5 hours and infusions were maintained for a total of 26.5 hours until animal kill to allow for a 2.5-hour ischaemia period and 24-hour reperfusion period.

Muscle viability assay. Bilateral (tourniqueted and non-tourniqueted) gastrocnemius muscles were harvested. Immediately upon harvest, a 2,3,5-triphenyltetrazolium chloride (TTC; MilliporeSigma, USA) stain was performed to assess muscle viability. TTC is a colourless, water-soluble dye that is reduced to a deep red, water-insoluble compound in the mitochondria of living cells. Gastrocnemius muscles were cut into 2 mm axial slices, cleaned with cold 0.9% sodium chloride solution, and incubated with a 1% TTC solution at room temperature for one hour. Slice images were digitalized and percentage muscle viability (dark red stain) was quantified using Adobe Photoshop (version 21.1, Adobe, USA) by two blinded male experimental personnel (DBB and DJK) whose quantifications were averaged. This stain has previously been used as a viability assay in murine skeletal muscle.[5] Contralateral, non-tourniqueted limbs were used as internal controls. No mice were excluded from TTC analysis.

Gene expression analyses. Bilateral (tourniqueted and non-tourniqueted) peroneal muscles were harvested. Gross specimens were stored in RNAlater (Qiagen, Germany) stabilization solution at -20°C until processing. RNA was isolated (RNeasy Kit; Qiagen), and genomic DNA was removed (RNase-free DNase set; Qiagen). The RNA was reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, USA), and quantitative real-time PCR was performed using the Applied Biosystems StepOnePlus detection system (Thermo Fisher Scientific) and RT^2 SYBR Green qPCR Mastermix (Qiagen) for eight samples per cohort. Specific primers for markers of cell death (CASP3) and inflammation (nitric oxide synthase 2 (NOS2), insulin-like growth factor 1 (IGF1), interleukin-1 beta (IL1β), tumour necrosis factor alpha (TNFα)) were designed using the PrimerBank sequence.[18] All reactions were performed in triplicates. Whole triplicates were excluded if one of the triplicates was a significant outlier.

Serum analyses. Immediately prior to the mice being killed, whole blood was collected via orbital venipuncture. Blood was allowed to clot for 30 minutes at room temperature and then centrifuged at 2,000 ×g for ten minutes at 4°C. The resulting supernatant (serum) was immediately collected and stored at -80°C until analysis.

To evaluate the extent of both muscle damage and kidney injury following the tourniquet-induced IR injury, circulating serum creatine kinase activity and creatinine, respectively, were quantified. Both creatine kinase activity and creatinine were quantified using commercially

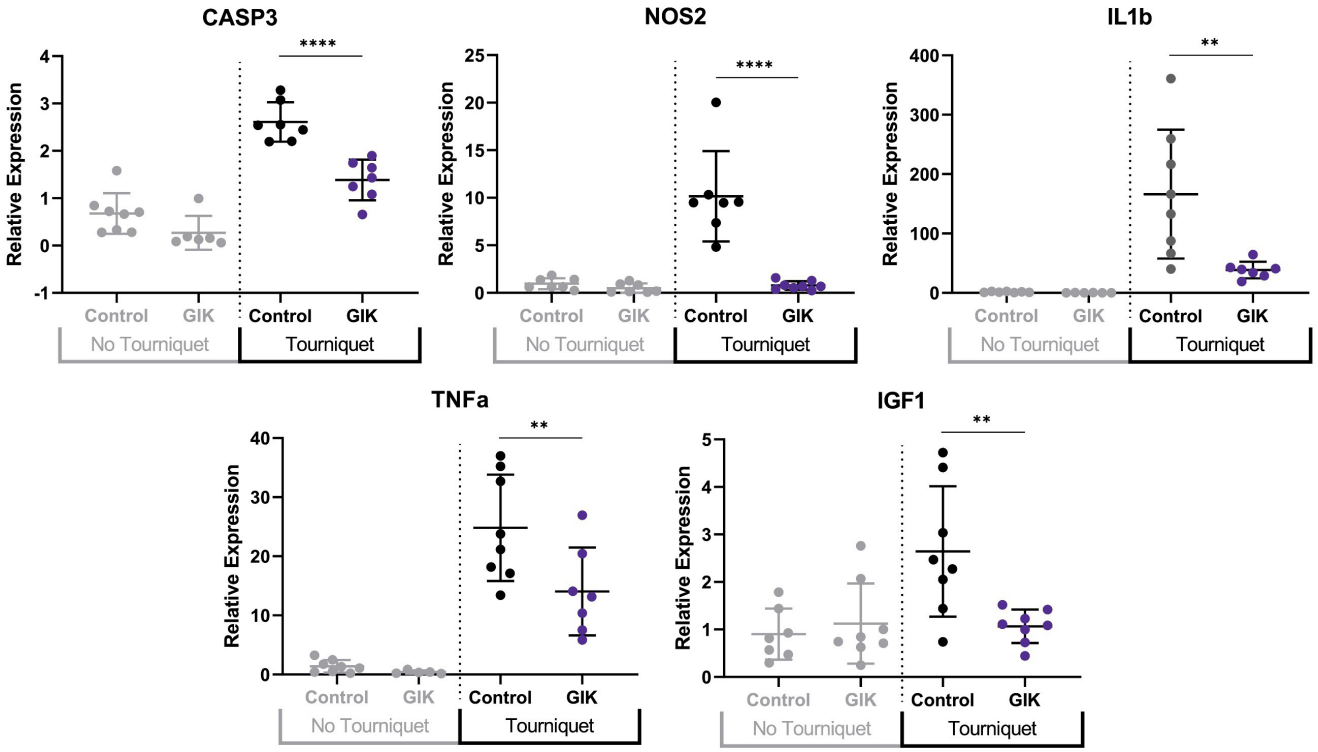

Fig. 2

Quantification of relative gene expression in tourniqueted peroneal muscles for the control cohort compared to the glucose-insulin-potassium (GIK) cohort demonstrating significantly greater cell death (CASP3: 2.611 (n = 7) vs 1.386 (n = 7), p < 0.001) and significantly more inflammation (nitric oxide synthase 2 (NOS2): 10.150 (n = 7) vs 0.772 (n = 7), p < 0.001; interleukin-1 beta (IL-1ß): 166.300 (n = 8) vs 38.500 (n = 7), p = 0.002; tumour necrosis factor alpha (TNF-a): 24.820 (n = 8) vs 14.060 (n = 7), p = 0.010; insulin-like growth factor 1 (IGF1): 2.643 (n = 8) vs 1.066 (n = 8), p = 0.007, one-way analysis of variance with Tukey's multiple comparisons). **p ≤ 0.01; ****p ≤ 0.001.

available colorimetric assays (Creatine Kinase Activity Assay Kit, ab155901; Abcam, UK; and Creatinine Assay Kit, ab65340; Abcam) performed on a FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices, USA). Serum was collected from five mice and all assays were performed in triplicate. Whole triplicates were excluded if one of the triplicates was a significant outlier.

Pain score analysis. Prior to being killed, all animals were observed for five minutes using a previously reported, standardized pain score ethogram.[19] The ethogram evaluates and assigns a value based on the condition of the animal's coat, eyes, posture/coordination, and overall condition. All mice were evaluated by two blinded male reviewers (DBB and DJK) and the two reviewers' scores were averaged. No animals were excluded from pain score analysis.

Statistical analysis. Muscle viability assay, gene expression analyses, serum analyses, and pain score analysis were compared and presented using means, standard deviations (SDs), and p-values. Statistical significances for muscle viability assay and gene expression analyses were calculated by one-way analysis of variance (ANOVA) with Tukey's multiple comparisons tests to calculate individual p-values for comparisons between the control and GIK-treated tourniqueted mice. Statistical significance for serum and pain score analyses were calculated using independent-samples *t*-tests. Significance was set to p ≤ 0.05. Statsitical analyses were performed using GraphPad Prism (version 8.3.0; GraphPad, USA).

Results

The use of GIK during a tourniquet-induced 2.5-hour ischaemia and 24-hour reperfusion period in a murine model resulted in significantly greater deep-red staining with TTC compared to saline control (GIK 22.07% (SD 15.48%) vs control 3.14% (SD 3.29%), p = 0.006, ANOVA with Tukey's multiple comparisons) (Figure 1). Given that TTC only stains living cells with active mitochondria, this finding strongly suggests that GIK protects skeletal muscle viability following tourniquet-induced IR injury.

Gene expression analyses of tourniqueted peroneal muscle supports the finding that GIK is myoprotective and suggests a mechanism for its action. First, consistent with our TTC findings, CASP3, a marker of cell death, was significantly elevated in the saline control cohort compared to the GIK cohort (2.61 (SD 0.42) vs 1.39 (SD 0.43), p < 0.001, ANOVA with Tukey's multiple comparisons). Additionally, NOS2, IL1β, TNFα, and IGF1, all markers of inflammation, were significantly elevated in the saline control cohort compared to the GIK cohort (NOS2, 10.15 (SD 4.75) vs 0.77 (SD 0.45), p < 0.001; IL-1β, 166.30 (SD 108.40) vs 38.50 (SD 14.12), p =

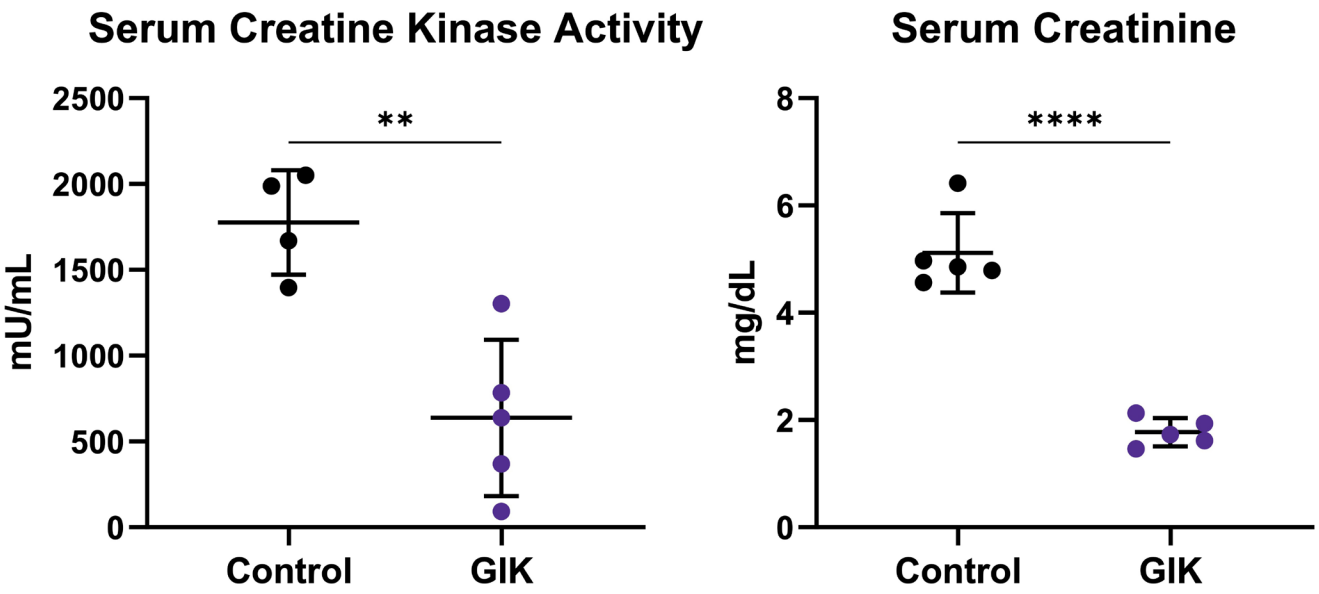

Fig. 3

Serum analysis of creatine kinase activity to quantify circulating skeletal muscle breakdown products and creatinine concentration as a marker of kidney injury. Both creatine kinase activity and creatinine concentration were significantly greater in the control cohort compared to the glucose-insulin-potassium (GIK) cohort (creatine kinase activity: 1,776 (n = 4) vs 637.7 (n = 5), p = 0.004; creatinine: 5.12 (n = 5) vs 1.78 (n = 5), p < 0.001, independent-samples *t*-test). **p ≤ 0.01; ****p ≤ 0.001.

0.002; TNF-α, 24.82 (SD 9.00) vs 14.06 (SD 7.44), p = 0.010; IGF1, 2.64 (SD 1.37) vs 1.07 (SD 0.35), p = 0.007, ANOVA with Tukey's multiple comparisons) (Figure 2). As hypothesized, it appears that the ability of GIK to protect against IR-related muscle death may be due to its anti-inflammatory properties. Notably, no differences in skeletal muscle viability or gene expression were found in the non-tourniqueted limbs between the treatment and control mice, suggesting that GIK is myoprotective against IR injury but does not alter the baseline characteristics of non-injured muscle (Figures 1 and 2).

Analysis of serum collected at the time of kill also supports these findings. Serum creatine kinase activity, a well-established serum marker of muscle damage,[20,21] was significantly greater in the saline control cohort compared to the GIK cohort (1,776.00 mU/ml (SD 303.30) vs 637.7 mU/ml (SD 455.80), p = 0.004, independent-samples *t*-test) (Figure 3). In combination with our TTC and gene expression findings, the ability of GIK to reduce circulating serum creatine kinase following tourniquet-induced IR injury suggests that it is myoprotective against IR injury.

Since elevated creatine kinase levels are associated with kidney injury,[22] we quantified serum creatinine levels, a gold-standard marker of kidney injury,[23] to look for signs of kidney injury and to strengthen the association between GIK and decreased muscle injury following IR injury. Similar to what we found for creatine kinase, serum creatinine was significantly greater in the saline control cohort compared to the GIK cohort (5.12 mg/dl (SD 0.74) vs 1.78 mg/dl (SD 0.26), p < 0.001, independent samples *t*-test) (Figure 3). Thus, the ability of GIK to protect skeletal muscle following tourniquet-induced IR injury as shown by increased TTC staining, decreased cell death and inflammatory gene expression, and decreased circulating serum creatine kinase, also protects against renal injury as shown by the decreased circulating serum creatinine.

Finally, using a published murine ethogram, GIK was associated with a significant reduction in IR-related pain compared to saline control (2.10/13 (SD 1.41/13) vs 6.00/13 (SD 3.06/31), p = 0.003, independent samples *t*-test) (Figure 4). While most of the pain relief was likely from a decrease in skeletal muscle death, a component of the lower pain ethogram score may be due to a lower renal and systemic morbidity resulting in less malaise.

Discussion

Systemically administered GIK is associated with greater skeletal muscle viability by TTC stain, lower skeletal muscle cell death and inflammation by gene expression analysis, lower skeletal muscle breakdown and kidney injury by serum analysis, and less pain by a murine ethogram.[19] Based on our understanding of GIK's mechanism of action in the cardiac literature,[8,9,15] the similar mechanisms of action by which skeletal and cardiac muscle undergo IR injury,[13,14] and our gene expression analyses, it appears that GIK functions by reducing the significant inflammatory response that occurs during an IR injury. These findings strongly support the hypothesis that GIK is myoprotective and can reduce systemic morbidity following tourniquet-induced IR injury in a murine model.

The myoprotective applicability of time-zero GIK administration is prophylactic in traumatic and elective tourniquet application scenarios. Our data suggest that

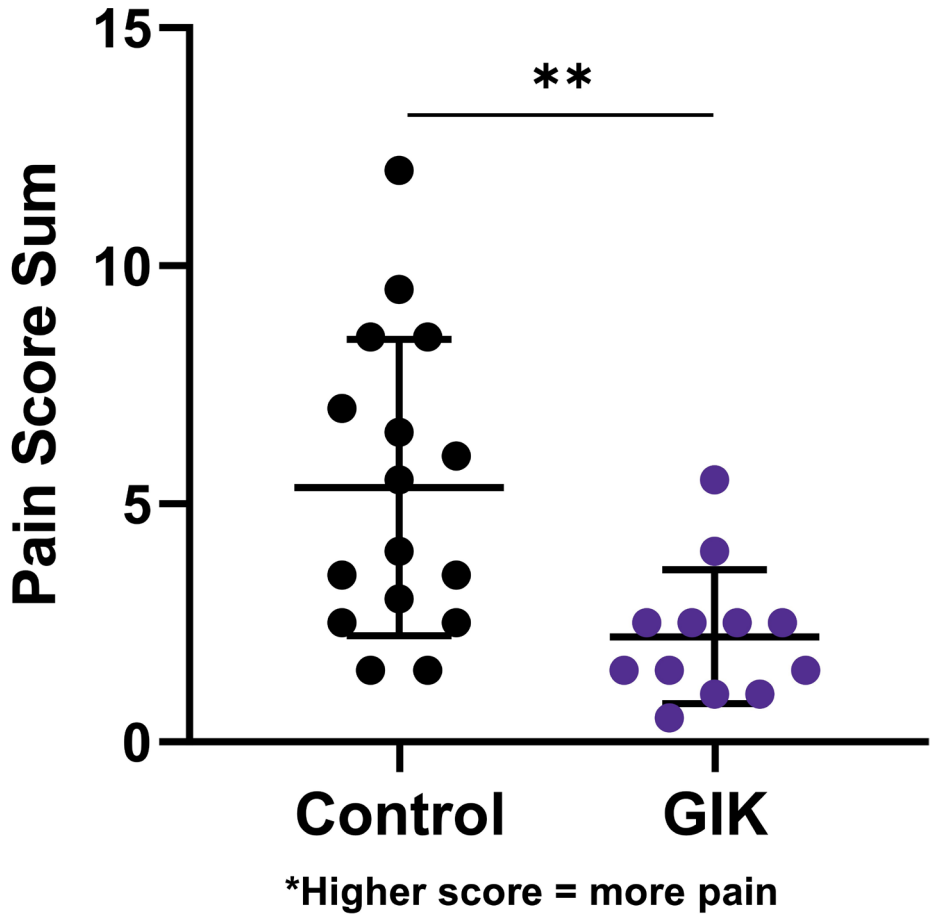

Fig. 4

Pain score analysis by murine ethogram calculated 24 hours after tourniquet release, demonstrating significantly less pain in the glucose-insulin-potassium (GIK) cohort compared to the control cohort (5.34/13 (n = 16) vs 2.21/13 (n = 12), p = 0.003, independent-samples *t*-test). A higher score represents more pain.[19] **p ≤ 0.01.

immediate GIK administration whenever a tourniquet is being used can decrease skeletal muscle damage and reduce systemic morbidity from ischaemia and delayed reperfusion. Whether the delayed administration of GIK following acute ischaemic injury is similarly myoprotective requires additional study.

To our knowledge, this is the first study evaluating the ability of GIK to reduce skeletal muscle death following IR injury. The only other publication studying GIK in skeletal muscle was performed in 1988 and showed that GIK maintains skeletal muscle glycogen during fasting.[12] As such, these data suggest the need for both basic science and translational studies that identify the specific mechanisms of action of GIK in skeletal muscle, and evaluate its clinical utility in larger animal models and in human studies.

In cardiac muscle, GIK appears to function by increasing glycogen and ATP, and reducing FFA and ROS.[8,9] In addition to these basic science findings, clinically it has been shown to have both a functional benefit, as shown by an increased postoperative cardiac index, and a mortality benefit.[10,11,24] Furthermore, while our data begin to define the utility and mechanism of action of GIK, its human clinical benefit for skeletal muscle injuries remains unclear.

There are several limitations to this study. Importantly, while a small animal model is one of the first steps in evaluating the clinical utility of a therapeutic treatment, findings in a small animal model do not always translate to similar findings in human trials. Further, using only male mice in this pilot study limits the generalizability of our findings. As such, large animal models and human trials that include both male and female subjects are required to confirm our findings. Additionally, it is important to study how GIK affects all the reported variables following IR injury at earlier and later timepoints. If GIK has a lasting effect that prevents chronic disability from skeletal muscle and renal injury, it could fundamentally alter the way we approach the treatment of musculoskeletal injury.

If future studies continue to support GIK, its clinical benefit would be immeasurable. We see prophylactic and therapeutic applicability for it following traumatic and elective tourniquet use, in the setting of compartment syndrome and significant musculoskeletal trauma, and for limb-related vascular surgery. Future work will test GIK in large animal models and humans to reduce skeletal

muscle IR injury in tourniquet use, and in the setting of compartment syndrome. Our findings strongly support the need for additional in vitro and in vivo trials to further define the role of GIK in the treatment of musculoskeletal injuries.

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Supplementary material

ARRIVE checklist.

References

1. **No authors listed**. WISQARS Cost of Injury: Number of Injuries and Associated Costs. Center for Disease Control Prevention. 2017. https://wisqars.cdc.gov/cost/ (date last accessed 23 February 2023).
2. **Mabry RL**. Tourniquet use on the battlefield. *Mil Med*. 2006;171(5):352–356.
3. **Inaba K**, **Siboni S**, **Resnick S**, **et al**. Tourniquet use for civilian extremity trauma. *J Trauma Acute Care Surg*. 2015;79(2):232–237; quiz 332-3.
4. **Welling DR**, **McKay PL**, **Rasmussen TE**, **Rich NM**. A brief history of the tourniquet. *J Vasc Surg*. 2012;55(1):286–290.
5. **Corrick RM**, **Tu H**, **Zhang D**, **et al**. Dexamethasone protects against tourniquet-induced acute ischemia-reperfusion injury in mouse hindlimb. *Front Physiol*. 2018;9:244.
6. **Kam PC**, **Kavanagh R**, **Yoong FF**. The arterial tourniquet: pathophysiological consequences and anaesthetic implications. *Anaesthesia*. 2001;56(6):534–545.
7. **Sodi-Pallares D**, **Testelli MR**, **Fishleder BL**, **et al**. Effects of an intravenous infusion of a potassium-glucose-insulin solution on the electrocardiographic signs of myocardial infarction. A preliminary clinical report. *Am J Cardiol*. 1962;9:166–181.
8. **Doenst T**, **Bothe W**, **Beyersdorf F**. Therapy with insulin in cardiac surgery: controversies and possible solutions. *Ann Thorac Surg*. 2003;75(2):S721–8.
9. **Schipke JD**, **Friebe R**, **Gams E**. Forty years of glucose-insulin-potassium (GIK) in cardiac surgery: a review of randomized, controlled trials. *Eur J Cardiothorac Surg*. 2006;29(4):479–485.
10. **van der Horst ICC**, **Zijlstra F**, **van't Hof AWJ**, **et al**. Glucose-insulin-potassium infusion inpatients treated with primary angioplasty for acute myocardial infarction: the glucose-insulin-potassium study: a randomized trial. *J Am Coll Cardiol*. 2003;42(5):784–791.
11. **Fan Y**, **Zhang A-M**, **Xiao Y-B**, **Weng Y-G**, **Hetzer R**. Glucose-insulin-potassium therapy in adult patients undergoing cardiac surgery: a meta-analysis. *Eur J Cardiothorac Surg*. 2011;40(1):192–199.
12. **Reikerås O**, **Nordstrand K**, **Henden T**. Effects of fasting and glucose-insulin-potassium on glycogen contents in heart, skeletal muscle and liver. *Scand J Clin Lab Invest*. 1988;48(3):285–288.
13. **Carmeli E**, **Aizenbud D**, **Rom O**. How do skeletal muscles die? An overview. *Adv Exp Med Biol*. 2015;861:99–111.
14. **Buchalter DB**, **Kirby DJ**, **Egol KA**, **Leucht P**, **Konda SR**. Can lessons learned about preventing cardiac muscle death be applied to prevent skeletal muscle death? *Bone Joint Res*. 2020;9(6):268–271.
15. **Lopaschuk GD**, **Wambolt RB**, **Barr RL**. An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. *J Pharmacol Exp Ther*. 1993;264(1):135–144.
16. **Stanley AW Jr**, **Moraski RE**, **Russell RO**, **et al**. Effects of glucose-insulin-potassium on myocardial substrate availability and utilization in stable coronary artery disease. Studies on myocardial carbohydrate, lipid and oxygen arterial-coronary sinus differences in patients with coronary artery disease. *Am J Cardiol*. 1975;36(7):929–937.
17. **Turner PV**, **Brabb T**, **Pekow C**, **Vasbinder MA**. Administration of substances to laboratory animals: routes of administration and factors to consider. *J Am Assoc Lab Anim Sci*. 2011;50(5):600–613.
18. **No authors listed**. Primer search. The Massachusetts General Hospital. 2006. http://pga.mgh.harvard.edu/primerbank/ (date last accessed 8 February 2023).
19. **Clark MD**, **Krugner-Higby L**, **Smith LJ**, **Heath TD**, **Clark KL**, **Olson D**. Evaluation of liposome-encapsulated oxymorphone hydrochloride in mice after splenectomy. *Comp Med*. 2004;54(5):558–563.
20. **Chiu D**, **Wang HH**, **Blumenthal MR**. Creatine phosphokinase release as a measure of tourniquet effect on skeletal muscle. *Arch Surg*. 1976;111(1):71–74.
21. **Smith RM**, **Neuman TS**. Elevation of serum creatine kinase in divers with arterial gas embolization. *N Engl J Med*. 1994;330(1):19–24.
22. **Brown CVR**, **Rhee P**, **Chan L**, **Evans K**, **Demetriades D**, **Velmahos GC**. Preventing renal failure in patients with rhabdomyolysis: do bicarbonate and mannitol make a difference? *J Trauma*. 2004;56(6):1191–1196.
23. **Bellomo R**, **Kellum JA**, **Ronco C**. Acute kidney injury. *Lancet*. 2012;380(9843):756–766.
24. **Bothe W**, **Olschewski M**, **Beyersdorf F**, **Doenst T**. Glucose-insulin-potassium in cardiac surgery: a meta-analysis. *Ann Thorac Surg*. 2004;78(5):1650–1657.

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- U. Anil: Conceptualization, Methodology, Writing – original draft, Writing – review & editing.
- S. R. Konda: Conceptualization, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing.
- P. Leucht: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

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