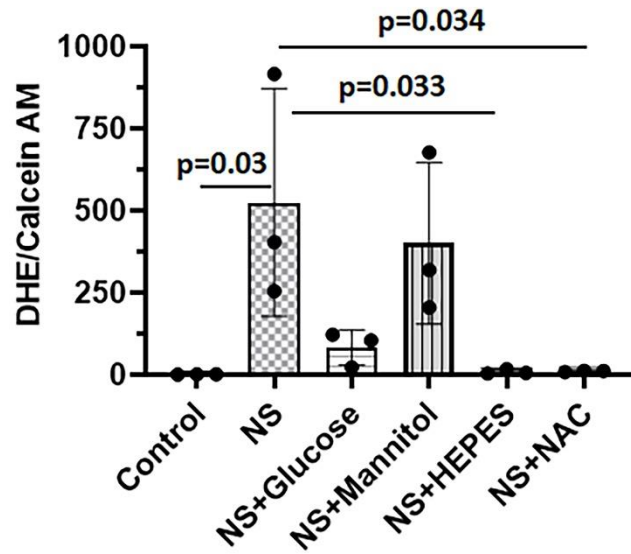


Supplementary Figure S1. Effect of normal saline (NS) and buffers (25 mM HEPES or 0.5 mM sodium bicarbonate) on chondrocyte death using a flow cytometer. (a-c) Representative plots for annexin V (FL1-H; x-axis)/propidium iodide (PI) (FL3-H; y-axis) staining after irrigant incubation for 30 minutes (a), 1 hour (b), and 3 hours (c). Q1: annexin V negative/PI positive, Q2: annexin V positive / PI positive, Q3: annexin V positive / PI negative, Q4: annexin V negative / PI negative. (d-f) Representative FSC vs SSC plots for cell morphology after irrigant incubation for 30 minutes (d), 1 hour (e), and 3 hours (f). FSC-H (forward scatter height; x-axis) and SSC-H (side scatter height; y-axis) represent cell size and granularity, respectively.



Supplementary Figure S2. Effect of 0.9% normal saline (NS) and various supplements on oxidative stress in monolayer primary bovine chondrocytes. Glucose (Sigma-Aldrich): 31.52 mM, Mannitol (Research Products International): 31.52 mM, HEPES: 25 mM, NAC (Sigma-Aldrich): 2.5 mM with 26 mM sodium bicarbonate (Research Products International) (n = 3).

Supplementary Table S1. List of metabolites showing ≥ 2 -fold changes between control and 0.9% normal saline (NS). Chondrocytes were treated with each irrigation solution for 30 minutes (n = 6). Positive values of fold change (up): NS or NS + HEPES > control, negative values of fold change (down): NS or NS + HEPES < control.

| | NS <i>versus</i> Control | | NS + HEPES <i>versus</i> Control | |
|---|--------------------------|---------|----------------------------------|---------|
| Metabolites (up) | Fold change | p-value | Fold change | p-value |
| Glucose 6-phosphate | 70.3 | 5.9E-09 | 23.4 | 1.5E-04 |
| Ribose 5-phosphate | 34.3 | 5.8E-09 | 6.8 | 1.2E-02 |
| Fructose 6-phosphate | 7.5 | 5.9E-09 | 2.8 | 7.6E-04 |
| O-octanoyl-R-carnitine | 5.0 | 1.1E-07 | 7.0 | 6.2E-09 |
| D-Ribulose 5-phosphate | 4.2 | 5.8E-09 | 2.0 | 1.6E-05 |
| O-propanoyl-carnitine | 4.2 | 1.9E-08 | 3.9 | 6.1E-08 |
| Inosine | 3.2 | 5.8E-09 | 0.6 | 4.8E-03 |
| Mannose | 3.0 | 6.3E-08 | 1.9 | 6.8E-04 |
| Guanosine | 2.9 | 5.9E-09 | 1.7 | 1.2E-04 |
| Adenosine monophosphate (AMP) | 2.3 | 5.8E-09 | 0.8 | 9.1E-03 |
| Nicotinamide adenine dinucleotide phosphate (NADP+) | 2.2 | 7.7E-08 | 1.7 | 8.1E-05 |
| Deoxyguanosine diphosphate (dGDP) | 2.2 | 5.8E-09 | 0.6 | 3.3E-05 |
| O-hexanoyl-R-carnitine | 2.1 | 2.7E-08 | 2.1 | 2.3E-08 |
| Metabolites (down) | Fold change | p-value | Fold change | p-value |
| Reduced nicotinamide adenine dinucleotide phosphate (NADPH) | -31.1 | 6.6E-09 | -0.8 | 7.2E-04 |
| Alpha-Ketoglutarate (KG) | -22.0 | 5.8E-09 | -15.5 | 5.8E-09 |
| Alpha-Ketoisovalerate (KIV) | -12.4 | 6.1E-09 | -4.8 | 8.3E-09 |
| Cytidine triphosphate (CTP) | -11.8 | 5.8E-09 | -1.4 | 5.7E-06 |
| Alpha-Keto-beta-Methylvalerate (KMV) | -10.2 | 5.8E-09 | -10.2 | 5.8E-09 |
| Nicotinamide adenine dinucleotide + hydrogen (NADH) | -9.6 | 1.2E-07 | -0.8 | 7.0E-03 |
| O-oleoylcarnitine | -9.1 | 1.2E-05 | -1.0 | 9.8E-01 |
| Alpha-Ketoisocaproate (KIC) | -9.0 | 5.8E-09 | -6.8 | 5.8E-09 |
| Lactate | -7.2 | 5.8E-09 | -3.3 | 5.8E-09 |
| Xanthosine | -5.7 | 0.020 | -0.6 | 4.4E-02 |
| Pyruvic acid | -5.5 | 5.8E-09 | -2.6 | 6.4E-09 |
| Cytidine diphosphate (CDP) | -4.8 | 5.8E-09 | -2.2 | 5.8E-09 |
| Oxidized glutathione (GSSG) | -4.8 | 7.9E-08 | -1.7 | 1.4E-04 |
| O-Phosphoethanolamine | -4.4 | 7.1E-09 | -3.6 | 9.1E-09 |

| | | | | |
|--|------|---------|------|---------|
| Dihydroxyacetone phosphate (DHAP) | -4.1 | 5.8E-09 | -2.2 | 5.8E-09 |
| Beta-Hydroxybutyrate (3-Hydroxybutyrate) | -3.8 | 5.8E-09 | -2.3 | 5.9E-09 |
| Inosine monophosphate (IMP) | -3.8 | 1.4E-07 | -1.8 | 8.2E-05 |
| Malate | -3.7 | 1.7E-08 | -7.3 | 6.9E-09 |
| Citrulline | -3.7 | 5.8E-09 | -3.6 | 5.8E-09 |
| Deoxythymidine triphosphate (dTTP) | -3.6 | 7.4E-07 | -2.4 | 9.4E-06 |
| Succinic acid | -3.5 | 8.1E-09 | -4.2 | 6.7E-09 |
| Guanosine triphosphate (GTP) | -3.2 | 6.0E-09 | -0.9 | 4.5E-01 |
| Hypoxanthine | -3.1 | 4.8E-07 | -1.0 | 0.838 |
| Fumarate | -3.0 | 8.3E-08 | -3.7 | 2.7E-08 |
| Glutarylcarntine | -3.0 | 5.9E-09 | -1.8 | 2.8E-08 |
| Pyruvate | -2.9 | 5.8E-09 | -2.0 | 5.9E-09 |
| Adenosine diphosphate (ADP) ribose | -2.9 | 6.1E-09 | -1.6 | 6.3E-07 |
| Carnitine | -2.9 | 5.8E-09 | -1.4 | 1.7E-08 |
| Lysine | -2.8 | 5.8E-09 | -2.3 | 5.8E-09 |
| Malic acid | -2.8 | 1.8E-06 | -4.0 | 2.3E-07 |
| Fumaric acid | -2.8 | 2.9E-06 | -4.1 | 3.4E-07 |
| Uridine triphosphate (UTP) | -2.5 | 5.8E-09 | -1.0 | 0.506 |
| Deoxyadenosine triphosphate (dATP) | -2.4 | 5.8E-09 | -1.6 | 2.2E-08 |
| Citric acid | -2.4 | 5.7E-04 | -2.5 | 4.8E-04 |
| Deoxyguanosine triphosphate (dGTP) | -2.1 | 6.2E-09 | -0.9 | 3.6E-04 |