Supplementary Materials for

Lack of nutritional immunity in diabetic skin infections promotes *Staphylococcus aureus* virulence

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- Figs. S1 to S10
- Table S1
Figure S1

A. Percent Initial Weight

B. Log_{10} CFU/Liver

C. Log_{10} CFU/Heart

p < 0.0001 for all panels.
Figure S2

A. Untreated LPS
B. "GLUT-3" Unt LPS Glc- + LPS
C. GLUT-3
D. STZ

E. DHR: AU

F. Percent Mo\(^{\text{+}}\) killing

\( p = 0.0214 \)
Figure S3

A. GLUT-1

WT

GLUT-1

B. Y-NO₂

C. GLUT-1⁻/⁻

D. 

E. 

F. 

Log₁₀ CFU/Abcess

WT  GLUT-1

p = 0.0002

Log₁₀: CFU/Kidney

WT  GLUT-1

p = 0.0002
Figure S4

A. Percent Initial Weight

B. Log 10: CFU / Liver

C. Log 10: CFU / Heart

D. Untreated Day 3
E. STZ Day 3
F. Δagr STZ Day 3
Figure S5

A. Lesion area: mm²

B. Percent initial weight

C. Log 10: CFU/Abscess

D. Log 10: CFU/Kidney

E. Log 10: CFU/Liver

F. Log 10: CFU/Heart

N. S.
Figure S6

A. Metabolic pathway diagram showing the glycolysis and TCA cycle processes.

- Upper Glycolysis:
  - Glucose
  - Glucose-6-phosphate
  - Fructose-1,6-bisphosphate
  - DHAP
  - GAP
  - ATP

- Lower Glycolysis:
  - PEP
  - ATP
  - Pyruvate
  - Acetyl-CoA

- TCA Cycle:
  - Acetate

B. Graph showing the relative expression of glcU gene with statistical significance (p < 0.0001).

C. Graph showing the relative expression of glcB gene with statistical significance (p < 0.0001).

D. Graph showing the relative expression of glcA gene with statistical significance (p = 0.002).
Figure S7

A. α-Hla

B. p = 0.0110

C. p < 0.0001

D. p < 0.0001

E. p < 0.0001

F. p < 0.0001

G. p < 0.0001

H. p = 0.0003

I. p < 0.0001
Figure S8

A.  
![Graph showing percent initial weight for different groups.](image)

B.  
![Graph showing Log10 CFU/Kidney for different groups.](image)

C.  
![Graph showing Log10 CFU/Liver for different groups.](image)

D.  
![Graph showing Log10 CFU/Heart for different groups.](image)
Figure S9

A. Percent Initial Weight

B. Log 10: CFU / Kidney

C. Log 10: CFU / Heart

D. Log 10: CFU / Liver

E. ΔG4 STZ Day 3
Figure S10

A.

B.

C.
**Table S1**

| Strain | Description |
|--------|-------------|
| LAC    | Methicillin Resistant Clinical *S. aureus* Isolate |
| SF8300 | Methicillin Resistant Clinical *S. aureus* Isolate |
| COL    | Methicillin Resistant Clinical *S. aureus* Isolate |
| AR1038 | *S. aureus* LAC pDB59 |
| AR1048 | *S. aureus* COL pDB59 |
| AR1198 | *S. aureus* LAC ΔagrA::Tn |
| AR0776 | *S. aureus* SF8300 ΔpfkA |
| AR0891 | *S. aureus* LAC Δpyk::Er<sup>+</sup> |
| AR1698 | *S. aureus* LAC ΔackA::Km<sup>+</sup> |
| AR1290 | *S. aureus* LAC ΔG2: ΔgIC::Kn<sup>+</sup>, ΔgIC::Sp<sup>+</sup> |
| AR1297 | *S. aureus* LAC ΔG4: ΔgIC::Kn<sup>+</sup>, ΔgIC::Er<sup>+</sup>, ΔgIC::Sp<sup>+</sup>, ΔgIC::Tc<sup>+</sup> |
| AR1320 | *S. aureus* LAC ptsH-H15A |
| AR1321 | *S. aureus* LAC ptsH-H15A/Δglk::Tn |
| AR1356 | *S. aureus* Protease |

| Primer | Sequence | Use |
|--------|----------|-----|
| 16S Fwd | TGATCCTGGGCTCAGGATGA | Real-Time PCR |
| 16S Rev  | TTCGCTCGACTTGCATGTA | Real-Time PCR |
| Hla Fwd  | ACAATTTTAGAGGCCAACTGAT | Real-Time PCR |
| Hla Rev  | TCCCCAATTTGTGATTACCAT | Real-Time PCR |
| Aur Fwd  | GCGTAAAGCGTCTCCCTCTTTTC | Real-Time PCR |
| Aur Rev  | GTGATGGTGGATGGTCCACATTTC | Real-Time PCR |
| Psmα Fwd  | TATCAAAGCCTTAATCGAACAATTC | Real-Time PCR |
| Psmα Rev  | CCCCTCAAATAAGATGTTCAATATC | Real-Time PCR |
Figure S1: Hyperglycemic mice have increased bacterial dissemination to peripheral organs UT (n=9) and STZ (n=11) mice were infected with $10^7$ CFU of WT. STZ-treated mice infected with WT have significantly increased weight loss compared to untreated animals (A). Infection with WT in STZ-treated animals resulted in significantly increased bacterial dissemination to livers (B) and hearts (C) compared to similarly infected untreated mice.
Figure S2: STZ-treated mice do not express GLUT-3.

Hematoxylin and eosin stained day seven tissues from *S. aureus* infected normal (A) and streptozotocin (B) treated mice. There is increased dermonecrosis in the STZ treated infected tissues compared to normal mice (red lines). The formation of a subcutaneous abscess in the normal tissue indicates control of tissues necrosis. In the STZ treated tissue there is no abscess formation and necrosis is observed penetrating to the ribs (green arrows) The black arrows indicate the relative areas where staining for YNO$_2$, iNOS, GLUT-1 and GLUT-3 was performed. Immunohistochemistry (IHC) was performed on tissues from untreated and STZ-treated mice using antibodies against GLUT-3 (red) and counterstained with DAPI (blue). GLUT-3 is highly abundant in infected tissues from untreated mice (C) but is absent from infected tissues in STZ-treated mice (D). RAW 264.7 macrophages were stimulated with LPS (100 ng/ml) and INF$\gamma$ (20 ng/ml) in media containing glucose or without glucose. Dihydrorhodamine 123 was used to measure intracellular peroxynitrite formation as an indication of respiratory burst. Stimulated macrophages had robust respiratory burst in the presence of glucose, but almost no burst in the absence of glucose (E). *S. aureus* was incubated for one hour in the presence of RAW 264.7 macrophages that were either not stimulated in the presence of glucose, or stimulated with LPS and INF$\gamma$ in the presence or absence of glucose. Stimulated macrophages in the presence of glucose killed more *S. aureus* than the not stimulated control (F). Stimulated macrophages in the absence of glucose had a significant reduction in bacterial killing compared to macrophages with glucose (F).
Figure S3: GLUT-1 LysM/Cre mice develop more severe *S. aureus* infections. IHC performed on infected tissues from WT and GLUT-1 LysM/Cre mice showed that WT mice had robust levels of GLUT-1 (A) and Y-NO₂ (B) staining. Conversely, GLUT-1 LysM/Cre mice had virtually no signal for GLUT-1 (C) or Y-NO₂ (D). Wild-type C57BL6 mice (N=9) and GLUT-1 LysM/Cre mice (N=7) were infected with 1 X10⁷ CFU of *S. aureus* LAC. GLUT-1 LysM/Cre mice had significantly higher bacterial burdens in the abscess (E) and in the kidneys (F).
Figure S4: Increased virulence in hyperglycemic mice is mediated by Agr.

STZ-treated mice infected WT displayed increased weight loss (A), increased dissemination to the liver (B) and heart (C) compared to untreated mice. While isogenic Δagr mutants rarely disseminated from the abscess in untreated mice, these mutants were able to dissemination in STZ-treated animals, albeit at reduced levels compared to WT LAC (B & C). Representative photographs showing dermonecrotic lesions three days after infection from LAC WT infected untreated mouse (red arrows) (D), STZ-treated mouse (red arrows) (E), and a Δagr infected STZ-treated mouse (yellow arrow) (G). Untreated mice have smaller open lesions than STZ-treated hyperglycemic mice. The Δagr infected mice display a small subcutaneous abscess as shown by the small bump in the skin (Yellow arrow) that is not observable at seven days after infection. Photo credit: Lance Thurlow, University of Pittsburgh.
**Figure S5: Proteases and α-hemolysin are still essential for invasive infections in hyperglycemic mice.** Untreated and STZ-treated mice were infected $10^7$ WT, Δhla, or ΔPro mutants (n=5 for all conditions). STZ-treated mice infected with WT LAC had significantly larger lesion sizes compared to all other infection combinations. Normal and hyperglycemic mice infected with LAC Δhla did not have any lesions. Untreated mice infected with ΔPro had similar lesion sizes to untreated WT infected mice, but lesion sizes did not increase in hyperglycemic mice (A). STZ-treated mice infected with WT or ΔPro displayed increased weight loss compared to similarly infected untreated mice. STZ-treated mice infected with Δhla did not display a significant difference in weight loss compared to similarly infected untreated animals (B). Untreated mice infected with WT had similar bacterial burdens to the same mice infected with ΔPro, but significantly higher abscess burdens than untreated mice infected with Δhla. All STZ-treated mice had significantly higher abscess burdens than their untreated infected counterparts. There was no significant difference in abscess burdens between the hyperglycemic infected groups (C). STZ-treated mice infected with WT had significantly more bacterial dissemination to the kidneys than all other infection combinations (D). STZ-treated mice infected with Δhla and ΔPro displayed significantly increased bacterial dissemination to kidneys compared to similarly infected untreated mice. STZ-treated mice infected with WT had significantly more bacterial dissemination to the liver than all other infection combinations (D). STZ-treated mice infected with ΔPro displayed significantly increased bacterial dissemination to the liver compared to similarly infected untreated mice, but hyperglycemic mice infected with Δhla did
not (E). STZ-treated mice infected with WT had significantly more bacterial dissemination to the heart than all other infection combinations. STZ-treated mice infected with ΔPro displayed significantly increased bacterial dissemination to the heart compared to similarly infected untreated animals, but STZ-treated mice infected with Δhla did not (F).
Figure S6: *S. aureus* requires glycolysis for ATP production, AgrC activation and virulence factor production. *S. aureus* imports glucose through the dedicated glucose transporters GlcB, GlcU, GlcA, and GlcC. Genes for *glcB* and *glcU* (blue) are ubiquitous among all Staphylococcal species, whereas, *glcA* and *glcC* (green) are only found in *S. aureus*. Deletions in the annotated glycolytic genes, *pfkA* and *pyk*, and the overflow metabolism gene *ackA* were used in this study. The sensor kinase, AgrC, has a unique ATP binding domain that requires high levels of intracellular ATP for full activity. In the presence of glucose, *S. aureus* will use glycolysis and overflow metabolism to rapidly generate ATP to allow for full activation of the sensor kinase AgrC and subsequent phosphorylation of AgrA to induce transcription of several virulence factors (A). WT was grown (OD660 ≈ 5.0) in defined with glucose (PNG) or casamino acids (PNC) as a primary carbon source. Quantitative real-time PCR for α-hemolysin (*hla*), phenol soluble modulin-α (*psma*), and aureolysin (*aur*) was performed on RNA isolated from a sample from the appropriate OD660. Our results show significantly less transcript of *hla* (B), *psma* (C), and *aur* (D) in *S. aureus* grown in PNC compared to PNG (n=3).
Figure S7: Sugar transport is essential for production of ATP and α-hemolysin and invasive infection. WT, pshH-H15A (pshH<sup>+</sup>), and pshH-H15A/ΔglcK double mutant were grown in a chemically defined media (OD<sub>660</sub> ≈ 5.0) with a combination of glucose and casamino acids as carbons sources. Spent supernatants were used for western blot analysis, and an aliquot of bacteria were used for intracellular ATP analysis. Representative α-hemolysin western blot showing (from left to right) WT, pshH<sup>+</sup>, and pshH<sup>+</sup>/ΔglcK (A). Quantification of α-hemolysin western blots normalized to OD<sub>660</sub> (n=3) shows decreased toxin in the supernatants of LAC pshH<sup>+</sup> and pshH<sup>+</sup>/ΔglcK compared to WT (B). The pshH<sup>+</sup> and the pshH<sup>+</sup>/ΔglcK mutants had diminished intracellular ATP compare to WT when normalize to OD<sub>660</sub> (n=3) (C). Untreated and hyperglycemic mice were infected with 10<sup>7</sup> CFU of WT, pshH<sup>+</sup> or pshH<sup>+</sup>/ΔglcK (WT UT, n=5; WT STZ, n=6; pshH<sup>+</sup> UT, n=5 pshH<sup>+</sup> STZ, n=7; pshH<sup>+</sup>/ΔglcK UT, n=5; pshH<sup>+</sup>/ΔglcK STZ, n=6). STZ-tretaed mice infected with WT had significantly larger lesions (A), and displayed significantly more weight loss than all of the other infection combinations (G). Bacterial burdens in the abscess were significantly higher in untreated mice infected with WT compared to the pshH<sup>+</sup> and pshH<sup>+</sup>/ΔglcK (F). The bacterial burden in abscess in hyperglycemic mice infected with WT was significantly higher than all other infection combinations (F). STZ-treated mice infected with the pshH<sup>+</sup> or pshH<sup>+</sup>/ΔglcK had significantly higher abscess burdens than in similarly infected untreated mice (F). STZ-treated mice infected with WT had significantly increased bacterial dissemination to the kidneys (E), the liver (I), and the heart (H) than similarly treated mice infected with the pshH<sup>+</sup> or pshH<sup>+</sup>/ΔglcK.
Figure S8: AckA is required for full virulence in hyperglycemic infections.

Untreated and STZ-treated mice were infected with $10^7$ CFU of WT ΔackA (WT UT, n=10; WT STZ, n=7; ΔackA UT, n=10; ΔackA STZ, n=11). WT STZ mice display significant weight loss compared to WT UT and ΔackA STZ (A). There is significantly more bacterial dissemination to the kidneys in hyperglycemic mice infected with WT and ΔackA compared to their untreated counterparts (B). There is significantly increased dissemination to kidneys in hyperglycemic mice infected with WT compared to ΔackA (B). There is significantly more bacterial dissemination to the liver in hyperglycemic mice infected with WT and ΔackA compared to their untreated infected counterparts. There is significantly increased dissemination to the liver in hyperglycemic mice infected with WT compared to ΔackA (C). There is significantly more bacterial dissemination to the heart in hyperglycemic mice infected with WT and ΔackA compared to their untreated infected counterparts. There is significantly increased dissemination to heart in hyperglycemic mice infected with WT compared to ΔackA (D).
**Figure S9: Glucose transporters are essential for invasive infections in hyperglycemic mice.** Untreated and STZ-treated mice were infected with $10^7$ CFU of WT or ΔG4 (WT UT, n=10; WT STZ, n=8; ΔG4 UT, n=10; ΔG4 STZ, n=12).

Hyperglycemic mice infected with WT displayed significant weight loss compared with LAC ΔG4 UT (A). Hyperglycemic mice infected with WT and ΔG4 had significantly higher dissemination to the kidneys compared to similarly infected untreated mice (B). Hyperglycemic mice infected with WT and ΔG4 had significantly increased bacterial dissemination to the liver compared to similarly infected normal mice (C). Hyperglycemic mice infected with WT and ΔG4 had significantly increased bacterial dissemination to the heart compared to similarly infected normal mice (D). A representative picture of a ΔG4 abscess (yellow arrow) three days after infection is comparable to a ΔagrA abscess at day three (Figure S2E) (E). Photo credit: Lance Thurlow, University of Pittsburgh.
**Figure S10:** The glucose transporters unique to *S. aureus*, GlcA and GlcC, are essential for full virulence potential in hyperglycemic mice.

Untreated and STZ-treated mice were infected with $10^7$ CFU of WT or ΔG2 (WT UT, n=10; WT STZ, n=8; ΔG2 UT, n=10; ΔG2 STZ, n=11) and euthanized after seven days of infection. Hyperglycemic mice infected with WT displayed significant weight loss compared WT UT and ΔG2 UT, as well as hyperglycemic mice infected with ΔG2 (F). Hyperglycemic mice infected with WT had significantly increased bacterial dissemination to the liver and the heart compared to all other combinations (G and H). Hyperglycemic mice infected with ΔG2 had increased bacterial dissemination to the liver (G) and heart (H) compared to similarly infected untreated mice, but significantly less than WT infected hyperglycemic animals.