Diabetes downregulates the antimicrobial peptide psoriasin and increases *E. coli* burden in the urinary bladder

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Diabetes is known to increase susceptibility to infections, partly due to impaired granulocyte function and changes in the innate immunity. Here, we investigate the effect of diabetes, and high glucose on the expression of the antimicrobial peptide, psoriasin and the putative consequences for *E. coli* urinary tract infection. Blood, urine, and urine exfoliated cells from patients are studied. The influence of glucose and insulin is examined during hyperglycemic clamps in individuals with prediabetes and in euglycemic hyperinsulinemic clamped patients with type 1 diabetes. Important findings are confirmed in vivo in type 2 diabetic mice and verified in human uroepithelial cell lines. High glucose concentrations induce lower psoriasin levels and impair epithelial barrier function together with altering cell membrane proteins and cytoskeletal elements, resulting in increasing bacterial burden. Estradiol treatment restores the cellular function with increasing psoriasin and bacterial killing in uroepithelial cells, confirming its importance during urinary tract infection in hyperglycemia. In conclusion, our findings present the effects and underlying mechanisms of high glucose compromising innate immunity.

The high prevalence of diabetes is a major global health challenge, often accompanied with increased risk of bacterial infections. In particular, *E. coli* urinary tract infections (UTI) are common and more frequently associated with serious complications, such as urosepsis. Traditionally, glycosuria is believed to provide an optimal environmental condition for bacterial growth. However, several factors like age, poor metabolic control, long term complications like neuropathy with incomplete bladder emptying as well as diabetic nephropathy contribute to the risk for UTI. Moreover, immunogenic impairment like reduced migration and chemotaxis of leucocytes play a role in the severe UTI pathogenesis of diabetic patients.

The host cells are well equipped with defense mechanisms shielding against invading microorganisms. In recent time, several factors have been identified that can protect the bladder from invading pathogens. Antimicrobial peptides (AMPs), part of first line innate immune response, defend the urothelium from pathogens and have the potential to become new UTI therapies. They are expressed by

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epithelial and endothelial cells as well as immune cells like neutrophils. We and others have previously reported the impact of human antimicrobial peptides cathelicidin, hBD1, hBD2 and RNase7 in the urinary tract. Most of these AMPs are cationic in nature and active against a wide range of both Gram-positive and Gram-negative bacteria. The antimicrobial peptide psoriasin, encoded by S100A7 is a

Fig. 1 | High glucose downregulates expression of psoriasin in plasma and urinary bladder. a Expression of S100A7 mRNA in urinary cells of patients with diabetes (DM), (n = 36) and non-diabetic individuals (n = 20) (unpaired two-tailed t test, p = 0.0164). b Urinary psoriasin normalized to urine creatinine (Ucr), in DM (n = 18) and non-diabetic individuals (n = 9) (unpaired two-tailed t test, p = 0.0094). c Schematic presentation of hyperglycemic clamps in prediabetes (n = 19), plasma psoriasin analysis before and 2 h after i.v. glucose infusion (paired two-tailed t test, p = 0.0264). d Schematic presentation of euglycemic, 5 mM, hyperinsulinemic clamp in type 1 diabetes (TID) (n = 23) and healthy (n = 18) individuals, plasma psoriasin analysis before and after insulin treatment (paired two-tailed t test). Expression of e S100a7a mRNA (unpaired two-tailed t test, p = 0.0044) and f protein in PBS treated diabetic, db/db (n = 13; n = 7) and non-diabetic, C57BL/6j (n = 11; n = 7) mice respectively (Mann-Whitney two-tailed test, p = 0.0204). Expression of g S100a7a mRNA (unpaired two-tailed t test, p = 0.0017) and h protein in 24 h E. coli infected, db/db (n = 7; n = 6) and C57BL/6j (n = 7; n = 6) respectively (Mann-Whitney two-tailed test, p = 0.0493). i S100A7 mRNA 5 (n = 6 infected; n = 8 uninfected), 15 (n = 6 infected; n = 6 uninfected), 30 (n = 6 infected; n = 6 uninfected), 60 (n = 6 infected; n = 6 uninfected) and 120 mins (n = 6 infected; n = 6 uninfected) post E. coli infection at MOI 10 or medium only after 24 h glucose treatment (normal = 6 mM: high=30 mM) of TERT-NHUC uroepithelial cells, compared to time 0 (n = 2), mean value is presented (unpaired two-tailed t test, p = 0.0072 and One-way ANOVA, multiple comparison test, p ≤ 0.05, p ≤ 0.01 respectively). j Intracellular psoriasin levels (mean fluorescence intensity, MFI, flowcytometry) after 36 h glucose treatment of TERT-NHUC, (n = 4) (Mann-Whitney two-tailed test, p = 0.0202). k Secretion of psoriasin after 24 h treatment with normal and high glucose of TERT-NHUC cells (n = 6) (unpaired two-tailed t test, p = 0.0165). In vitro experiments were performed in duplicate or triplicate with at least 3 independent experiments, presented as mean ± SEM, statistical outliers defined by Grubb's test were excluded. For in vivo and human material analysis, individual values and median are shown, *p < 0.05 and **p < 0.01. Source data are provided as a source data file.
member of the S100 protein family and has been detected in the urinary tract\textsuperscript{14}. It is mainly known for its high antibacterial activity against \textit{E. coli}, sequestering zinc which restricts the bacterial growth\textsuperscript{15}.

Although the importance of psoriasin during \textit{E. coli} infections is recognized, the possible activity in diabetes and during high glucose is not yet known. We here sought to investigate the impact of glucose on psoriasin and the pathogenesis of \textit{E. coli} UTI, with emphasis on the uroepithelium and defense strategies in the urinary bladder during diabetes.

**Results**

**High glucose decreases psoriasin in the serum/plasma and urinary bladder**

The possible impact of high glucose on antimicrobial peptides was analyzed in human uroepithelial cells TERT-NHUC and resulted in significantly lower expression of \textit{S100A7, DEFBI} and RNASE7, while \textit{CAMP, DEFBI} and \textit{DEFB03A} remained unchanged compared to low glucose (Fig S1a). Since \textit{DEFB03A}\textsuperscript{16} and RNASE7\textsuperscript{17} have previously been demonstrated to be compromised by high glucose, we focused on \textit{S100A7}, psoriasin. To ensure the clinical relevance, we investigated the psoriasin levels in patients with diabetes (Table S1). In these patients, we observed downregulation of \textit{S100A7} mRNA in urine exfoliated cells (Fig 1a) as well as lower psoriasin protein levels in urine (Fig 1b) with a similar trend of serum psoriasin levels compared to non-diabetic controls (Fig S1b).

To investigate the possible impact of glucose and insulin on psoriasin, hyperglycemic\textsuperscript{18} and euglycemic hyperinsulinemic clamps were used.\textsuperscript{19} Hyperglycemic clamps were performed in prediabetic individuals with a high risk of developing type 2 diabetes (T2D). In these clamp studies, glucose infusion for \textit{2 h} resulted in increased median plasma glucose from 5.4 (range 4.6–6.4) mM to 11.4 (range 10.8–12.5) mM. This stimulated insulin secretion, augmenting median plasma insulin levels from 16.1 (range 8.4-74.2) mU/L to 95.2 (range 35.6–160.0) mU/L. These changes were accompanied with decreased psoriasin levels (Fig 1c). Contrary, euglycemic hyperinsulinemic clamps in patients with type 1 diabetes (T1D) and in non-diabetic individuals\textsuperscript{20}, showed no difference in the plasma psoriasin levels (Fig 1d). Hence, the lower plasma psoriasin was associated with enhanced blood-glucose levels, but not with insulin levels.

To confirm our results in vivo, female db/db mice with T2D and C57BL/6j non-diabetic control mice were studied. The \textit{S100a7} mRNA (Fig 1e) and psoriasin protein levels (Fig 1f), were significantly lower in diabetic mouse bladders, with the peptide primarily localized in the superficial umbrella cell layers. To further investigate the effect on psoriasin during infection in diabetes, mice were transurethrally infected with \textit{E. coli}. We observed lower expression of \textit{S100a7} mRNA at the mRNA (Fig 1g), and protein levels (Fig 1h) at 24 h and 7 days post infection (Fig S1c, d) in urinary bladders of diabetic compared to control mice.

Similarly, TERT-NHUC uroepithelial cells cultured under normal glucose condition showed a rapid increase of \textit{S100A7} mRNA peaking already after 15 min of \textit{E. coli} infection (Fig 1i). On the other hand, TERT-NHUC cells cultured in high glucose expressed lower \textit{S100A7} mRNA (Figs. 1j and S1e), with similar results in 5637 uroepithelial cells (Fig S1f). In line with our mRNA data, lower cytoplasmic and vesicle associated psoriasin (Figs. 1j and S1g) as well as secreted psoriasin (Fig 1k) protein were also observed in high glucose treated TERT-NHUC cells.

**High glucose impairs IL-6 mediated psoriasin expression**

Since cytokines are known to regulate antimicrobial peptides\textsuperscript{20}, we hypothesized that not only psoriasin, but also cytokines could be compromised by high glucose. In line with this, the proinflammatory cytokines, \textit{IL1B} (Fig 2a) and \textit{IL6} (Fig 2b) were downregulated on the mRNA level in urine exfoliated cells from patients with diabetes. Moreover, the protein levels of IL-1\beta and IL-6 were lower and located in the superficial umbrella cells (Fig 2c) in diabetic mice bladders 24 h post \textit{E. coli} infection. Likewise, in high glucose treated TERT-NHUC uroepithelial cells, the expression of \textit{IL1B} and \textit{IL6} mRNA (Fig 2d, e) and IL-6 protein (Fig 2f) was compromised. Although, IL-6 is a known regulator of pSTAT3/STAT3, no difference of pSTAT3 was observed between high and low glucose treated TERT-NHUC cells substituted with 50 ng/ml of human IL-6 peptide (Fig 2g). In high glucose treated cells, the expression of SOCS3 mRNA, downstream of STAT3, was compromised (Fig 2h) possibly due to the increased expression of Aryl hydrocarbon receptor (AhR) mRNA in 5637 cells (Fig 2i), which is known to downregulate SOCS3\textsuperscript{21} and also to be upregulated by high glucose\textsuperscript{22}. Interestingly, SOCS3 mRNA was upregulated by IL-6 peptide treatment (Fig 2j) in TERT-NHUC cells. Further to confirm the effect of IL-1\beta on IL-6 expression, supplementation with 20 ng/ml IL-1\beta peptide resulted in increased expression of both IL-6 and \textit{S100A7} mRNA, which was quenched by diacerein, a specific IL-1\beta blocker (Fig 2k, l). IL-6 and psoriasin expressions have been shown to depend on each other in various cells\textsuperscript{23,24}. We therefore speculated that high glucose would lead to lower IL-6 levels which could affect psoriasin expression. Therefore, high glucose treated TERT-NHUC cells were treated with recombinant IL-6 peptide. We demonstrated that psoriasin was upregulated on both mRNA (Fig 2m) and protein levels (Fig 2n), confirming the interrelationship between IL-6 and psoriasin also during hyperglycemia.

**High glucose compromises occludin expression in plasma and urinary bladder**

Antimicrobial peptides have been shown to regulate epithelial barrier function\textsuperscript{25}. We therefore reasoned that the lower psoriasin levels may impact the expression of the tight junction protein, occludin. In line with this hypothesis, urine exfoliated cells from patients with diabetes, demonstrating lower levels of psoriasin, presented a clear downregulation of \textit{OCLN} (Fig 3a). Likewise, plasma from hyperglycemic clamped individuals with prediabetes, showed decreased occludin levels (Fig 3b).

Similarly, we observed lower \textit{Ocln} on the mRNA (Fig 3c) and protein levels in the diabetic vs non-diabetic mice, with the protein localized in the upper superficial umbrella cell layers of the bladder (Fig 3d), 24 h (Fig 3e, f) and 7 days (Fig S2a, b) post \textit{E. coli} infection. Our results were further confirmed in vitro, where uroepithelial cells, TERT-NHUC, exposed to high glucose down regulated occludin (Fig 3g) at the mRNA and protein levels (Fig 3h). To confirm the role of psoriasin in occludin expression, \textit{S100A7} was deleted in TERT-NHUC cells using the crispr/cas9 system which resulted in lower expression of occludin (Fig 3i) without inducing any adverse effect on cells as evident from nuclear and cytoskeleton integrity (Fig S2c). Furthermore, to verify the effect of psoriasin, TERT-NHUC uroepithelial cells treated with high glucose and supplemented with additional psoriasin peptide showed increased expression of \textit{OCLN} mRNA (Fig 3j). The Cys reduced form of psoriasin is a powerful endogenous zinc-chelator\textsuperscript{26}.

We therefore speculated that psoriasin could have a regulatory function in zinc homeostasis and its zinc-binding properties would impact the occludin gene expression\textsuperscript{27}. To test if the zinc-binding property of psoriasin could explain the observed effects, the cell penetrating and zinc specific chelator, N,N,N’,N’-tetrakis (2-pyridylmethyl) ethylene-diamine (TPEN) was used. Human uroepithelial cells, TERT-NHUC were treated with TPEN, followed by high glucose for a total 24 h. TPEN treatment resulted in increased \textit{OCLN} mRNA (Fig 3k) similar to the effect of the psoriasin peptide (Fig 3j) indicating a possible role of intracellular zinc depletion in the increased expression of \textit{OCLN} in high glucose treated human uroepithelial cells.

**High glucose and \textit{E. coli} infection modulate membrane proteins**

Since TERT-NHUC cells exposed to high glucose did not mount a pronounced psoriasin response when infected with \textit{E. coli}, we speculated that also other factors involved in bacterial infection could be
affected. Mannose or mannose-like receptors (MRC1) are known to play an important role in bacterial attachment to uroepithelial cells. In the uninfected state, expression of Mrc1 were similar in bladders of both types of mice. After 24 h of *E. coli* infection, however, diabetic but not non-diabetic control mice showed significantly higher expression of Mrc1 both on the mRNA (Fig. S2d) and protein levels (Fig. 4a). In line with these results, TERT-NHUC uroepithelial cells treated with high glucose and infected with *E. coli* showed increased expression of MRC1 at the mRNA (Fig. S2e) and protein levels (Fig. 4b). After bacterial attachment to uroepithelial cells, caveolin 1 influences the endocytic uptake of *E. coli*, by forming a flask shaped caveolae. Correspondingly, diabetic mice bladders showed clear upregulation of caveolin 1 (Fig. 4c), with similar result in high glucose treated TERT-NHUC uroepithelial cells (Fig. 4d). Based on our findings, we hypothesized that not only glucose, but possibly also psoriasin could impact membrane proteins. However, psoriasin peptide had no effect on MRC1 in TERT-
Fig. 2 | Effect of high glucose on cytokines and psoriasin expression. Expression of a IL1β (unpaired two-tailed t test) and b IL6 mRNA in urine cells of patients with diabetes (DM), (n = 36) and non-diabetic individuals (n = 20) (unpaired two-tailed t test, p = 0.0465). Expression of IL-1β and IL-6 (Mann-Whitney two-tailed test, p = 0.0075) expression in urinary bladders 24 h post E. coli infected diabetic, db/db mice (n = 5), and non-diabetic, C57BL/6j mice (n = 5). c IL1β and d IL6 (n = 9) (unpaired two-tailed t test, p = 0.0100, p ≤ 0.0001 respectively) mRNA level in TERT-NHUC uroepithelial cells cultured with normal and high glucose (normal = 6 mM; high = 30 mM) for 24 h. Secretion of IL-6 measured from TERT-NHUC after 24 h glucose treatment (n = 6) (unpaired two-tailed t test, p = 0.0036). g Flow cytometric analysis of pSTAT3 and total STAT3 (mean fluorescence intensity, MFI) after 36 h of glucose treatment and 1 h of 50 ng/ml of IL-6 peptide (n = 4) (One-way ANOVA, multiple comparison). h SOSC3 (n = 8), and i AKR (6 mM, n = 8; 30 mM, n = 7) mRNA level in TERT-NHUC and S637 revealed normal and high glucose for 24 h (unpaired two-tailed t test, p = 0.0009, p = 0.0101), respectively. J Expression of SOCS3 mRNA in high glucose and IL-6 0.06 peptide (50 ng/ml) treated TERT-NHUC cells after 24 h (unpaired two-tailed t test, p < 0.0001), (30 mM, n = 7; 30 mM+IL-6, n = 6). Expression of 5 IL6 (n = 10) and c S100A7 (30 mM and 30 mM+IL-1β, n = 10; 30 mM+Diacerein-IL-1β, n = 9) mRNA in high glucose and IL-1β peptide (20 ng/ml) in diacerein (50 µM) pretreated TERT-NHUC cells after 24 h (One-way ANOVA, multiple comparison, p < 0.05, p = 0.01 and p ≤ 0.001 respectively). mRNA expression of S100A7 (30 mM, n = 7; 30 mM+IL-6, n = 6) (unpaired two-tailed t test, p < 0.0001), n and psoriasin protein levels in high glucose and IL-6 0.06 peptide (50 ng/ml) treated TERT-NHUC cells (Mann-Whitney two-tailed test, p = 0.0075) after 24 h and 36 h respectively (n = 5). In vitro experiments were performed in either duplicate or triplicate with at least 3 independent experiments, presented as mean ± SEM, statistical outliers defined by Grubb’s test were excluded. For in vivo and human material analysis, individual values and median is mentioned, p < 0.05, p < 0.01, **p < 0.001 and ***p < 0.001. Source data are provided as a source data file.

Discussion

We here demonstrate that high glucose levels compromised the innate immune response and impaired epithelial integrity. These findings may offer an explanation of the clinical observation that patients with poorly controlled diabetes have higher risk of recurrent UTI, acute pyelonephritis and urosepsis. Thus, confirming that impaired glycemic control may contribute to enhanced risk of infections.

Our current results demonstrate that high glucose inhibits plasma psoriasin in prediabetic individuals during hyperglycemic clamp. Lower serum levels of AMPs have however previously been observed in diabetic patients. While insulin is known to regulate the...
Fig. 3 | Effect of diabetes and high glucose on occludin. a Expression of OCLN mRNA in urine cells from non-diabetic \((n = 20)\) and diabetic patients, DM \((n = 36)\) (unpaired two-tailed t test, \(p = 0.0391\)). b Plasma occludin levels in individuals with prediabetes \((n = 20)\) before and 2 h after i.v. glucose infusion (paired two-tailed t test, \(p = 0.0003\)). Expression of c Ocln mRNA (unpaired two-tailed t test, \(p \leq 0.0001\)) and d protein in PBS treated diabetic, db/db \((n = 13; \ n = 5)\) and non-diabetic, C57BL/6j \((n = 11; \ n = 5)\) mice respectively (Mann-Whitney two-tailed test, \(p = 0.0075\)). Expression of e Ocln mRNA (unpaired two-tailed t test, \(p = 0.0289\)) and f protein in 24 h E. coli infected db/db and C57BL/6j mice \((n = 7; \ n = 6)\) each respectively (Mann-Whitney two-tailed test, \(p = 0.0493\)). g Expression of OCLN mRNA level in TERT-NHUC uroepithelial cells cultured with glucose (normal = 6 mM; high = 30 mM) for 24 h \((n = 13)\) (unpaired two-tailed t test, \(p = 0.0059\)). h Representative microscopy image of occludin after 36 h glucose treatment \((n = 12)\) (Mann-Whitney two-tailed test, \(p = 0.0034\)). i Expression of psoriasin and occludin in S100A7 deleted \((ΔS100A7)\) TERT-NHUC cells, relative densitometry of psoriasin and occludin \((n = 8)\) are shown in comparison to control cells (Mann-Whitney two-tailed test, \(p = 0.0004\)). j OCLN mRNA after 24 h in high glucose and psoriasin (1600nM) peptide treated TERT-NHUC \((n = 6)\) (unpaired two-tailed t test, \(p = 0.0001\)). k OCLN mRNA post 2 h TPEN treatment in TERT-NHUC, followed by a total of 24 h with high glucose treatment \((n = 6)\) (unpaired two-tailed t test, \(p \leq 0.0001\)). In vitro experiments were performed in duplicate or triplicate with at least 3 independent experiments and presented as mean ± SEM, statistical outliers defined by Grubb’s test were excluded. For in vivo and human material analysis, median is mentioned. * \(p < 0.05\), ** \(p < 0.01\), *** \(p < 0.001\) and **** \(p < 0.0001\). Source data are provided as a source data file.
expression of RNase7 via the PI3K pathway, it is as evident from our clamp study results that insulin does not seem to exert any impact on psoriasin. We can therefore conclude that psoriasin is regulated by another pathway. This is further supported by the lack of influence by PI3K on the psoriasis expression in mammmary epithelial cells, MCF-10A, confirming differential influence of insulin on AMPs.

In line with our clinical findings from patients with diabetes, we also detected lower psoriasin levels in the urinary bladder of diabetic mice. Previously, lower β defensin-1 was reported in diabetic rats and mice. The importance of AMPs is evident as cathelicidin and β defensin-1 deficient mice are at greater risk for UTI. Interestingly, E. coli infection of high glucose treated uroepithelial cells failed to mount an increased expression of psoriasin. Likewise, other AMPs like β defensin-3 and cathelicidin were compromised in high glucose treated keratinocytes and in macrophages, respectively.

The effect of psoriasin is particularly interesting, since it is suggested to be the most potent and abundant E. coli-cidal AMP. RNase7 and cathelicidin, LL-37 are 10-fold less potent than psoriasin and cathelicidin is moreover less abundant in unstimulated epithelial cells, suggesting them less important relative to psoriasin in preventing E. coli infections. hBD-1 and mBD-1 are inactive as E. coli antimicrobials in their Cys-oxidized forms. Only the fully reduced, linearized form can kill E. coli. In addition to its superior effect on E. coli, psoriasin also has a high potency against Enterococcus sp, suggesting a broader protective role against infections.

Exfoliation of infected cells is a common host driven mechanism to eliminate infection, but also allows adhered bacteria from neighboring cells to infect deeper cell layers. Increased risk of infection is often associated with loss of barrier integrity, allowing bacterial invasion. Tight junction proteins, distributed on epithelial cells in the urinary tract, play a key role by protecting the deeper tissue from invading pathogens.

Our findings reveal lower occludin levels in individuals with prediabetes after glucose infusion as well as in patients with diabetes and in diabetic mice urinary bladders. These findings were associated with lower psoriasin levels. Similarly, high glucose has been reported to reduce the expression of occludin in human retinal endothelial cells and in the retina of diabetic mice. We demonstrate that uroepithelial cells exposed to high glucose restored the expression of occludin after treatment with psoriasin peptide. This is also in line with psoriasin...
mediated expression of occludin in human keratinocytes. In the non-diabetic situation, other AMPs like cathelicidin and β defensin-3 were reported to trigger the expression of occludin and claudins in human keratinocytes. Our result of restoration of OCLN mRNA expression in psoriasin supplemented high glucose treated uroepithelial cells further confirmed the vital role of psoriasin in maintenance of epithelial integrity in diabetes.

In diabetes, excessive advanced glycation end products are formed and accumulate in the bladder tissues which contribute to the attachment of uropathogenic E. coli (UPEC) and initiation of infection.
However, increased number of mannose containing binding sites for type 1 fimbriae has been demonstrated in diabetic mice urinary bladder. In the current study, we demonstrate upregulation of Mrc1 in infected diabetic but not in control mice, thereby facilitating bacterial colonization and invasion, further supporting the impaired ability to control bacterial infections.

Increased expression of caveolin 1 in diabetic mice bladder creates a favorable condition for bacterial entry. Our result highlights the importance of psoriasin mediated downregulation of caveolin 1 contributing to less bacterial uptake into the cell. Rearrangement of caveolin 1 further influences the cytoskeletal backbone of the cell, and the organization of actin filaments in turn facilitates the ability of bacteria to multiply within the cell. In addition, bacterial replication in less differentiated cell layers is inhibited by the denser actin network. However, we observed that high glucose concentration triggers the formation of more cortical actin which could potentiate the development of IBCs largely in the terminally differentiated umbrella cells of diabetic mice. High glucose is known to increase the
Fig. 6 | The impact of estradiol and high glucose on bacterial clearance. Bacterial load in a urine and b urinary bladders of non-diabetic, C57BL/6j (n = 8 each) and diabetic, db/db (n = 6, 8 mice respectively) after 24 h E. coli infection (unpaired two-tailed t test, p = 0.0003, p = 0.0001). e Representative mouse bladder sections stained for UPBlu and E. coli in C57BL/6j (n = 8) and db/db mice (n = 7). Lp, lamina propria; Lu, lumen; Ep., epithelium. Bacterial load in d urine and e urinary bladders of C57BL/6j (n = 5 and 6) and db/db (n = 6 in each group) after 14 days E. coli infection (unpaired two-tailed t test, p = 0.0242). f Impact of psoriasin peptide (5 μM) on E. coli survival in urine from type 2 diabetic patients, T2D (n = 20) and control individuals (n = 18) for 30 mins (paired two-tailed t test, p = 0.0273, p = 0.0442). g E. coli survival determined in lysate of uroepithelial TERT-NHUC cells treated with glucose (normal = 6 mM; high=30 mM) for 24 h and incubated with S100A7-specific monoclonal antibodies (α-S100A7) or isotype control (α-lgG) and compared to normal glucose levels (n = 4) (unpaired two-tailed t test, p = 0.0211).

Fig. 7 | Schematic representation of altered uroepithelial immune responses in high glucose. The impact of high glucose on uroepithelial cells, and the effects of psoriasin, IL-1β, IL-6 and estradiol on the background of high glucose exposed uroepithelial cells is demonstrated in the current study. Immunological changes occurring due to high glucose in comparison to a low glucose condition. b High glucose significantly downregulates psoriasin, IL-1β, IL-6, occludin, SOCS3 and RhoB without altering the pSTAT-3 level, but upregulates the expression of AHR. c Caveolin 1 with increased nuclear YAP/TAZ and cortical actin leading to increased bacterial load. E. coli infection further increases the expression of MRC1 in high glucose treated cells. d High glucose treated cells supplemented with IL-1β increases IL-6 and psoriasin. Supplementation of IL-6 increases psoriasin, SOCS3 and results in reduced nuclear YAP/TAZ. e Psoriasin peptide supplementation increases occludin and decreases caveolin 1 in high glucose treated cells. f Estradiol reverses the effect of high glucose and increases IL-6, psoriasin, cortical actin with reduced nuclear YAP/TAZ leading to increased intracellular bacterial killing even in high glucose treated cells.

amount of actin in pancreatic islets and to cause F-actin cytoskeleton rearrangement in podocytes. Intracellular filamentous actin reorganization resulted in translocation of YAP/TAZ from the cytoplasm to the nucleus. This reorganization is also regulated by caveolin 1, as reported in mouse embryonic fibroblast. Psoriasin expression is dependent on cell density and cell morphology, and nuclear YAP/TAZ significantly downregulates the expression of psoriasin in squamous cell carcinoma. Therefore, our observation of glucose mediated increased expression of caveolin 1, cortical actin formation and translocation of YAP/TAZ into nucleus emphasized a mechanistic pathway of psoriasin downregulation, confirmed in two different uroepithelial cells 5637 and TERT-NHUC. The Rho-family of small GTPases are master regulators of actin cytoskeleton rearrangements, and associated with enhanced formation of IBCs in human bladder epithelial cells, 5637. Our results support the findings of high glucose mediated RhoB downregulation and increased intracellular bacterial
load in diabetic mice. Further, we demonstrate RhoB influencing the translocation of nuclear YAP/TAZ.

The increased bacterial load and excessive IBCs detected in diabetic mice bladders even after 14 days of *E. coli* infection indicate a lack of ability to restrict bacterial growth and distribution compared to nondiabetic controls. Further, bacteria residing in the deeper tissues can act as reservoirs and contribute to future infection. Moreover, we demonstrate the role of high glucose mediated changes in the expression of psoriasin, occludin, Mrc1 and caveolin 1 resulted in increased bacterial load. Our result therefore further adds to the understanding of increased UPEC susceptibility in prediabetic and diabetic mice model and impaired UPEC clearance in diabetic mice.

Estradiol has recently been shown to increase glucose tolerance and insulin sensitivity in estrogen depleted ovariecotomized mice. It is also known to induce the mTOR pathway and actin polymerization through different molecular mechanisms. We here demonstrate the direct effect of estradiol on IL6 expression, a possible mechanism for an estradiol mediated psoriasis pathway without the involvement of IL1B in high glucose treated human uroepithelial cells. We further show that estradiol resulted in increased bacterial killing of *E. coli* infected uroepithelial cells, by translocating YAP/TAZ back to the cytoplasm and restoring the expression of psoriasin in high glucose treated uroepithelial cells without impacting the cytoskeleton. It has also been shown that estrogen receptor signaling affects psoriasis expression. Therefore, we believe that estradiol has an impact on regulating the immune response. Our observation confirms the beneficial effect of estrogen also during diabetic and high glucose conditions.

We conclude that hyperglycemia compromises psoriasin through the IL-6 and YAP/TAZ pathways affecting the epithelial barrier and causing cell membrane alterations. These changes further create a favorable condition for bacterial infection (Fig. 7). Our results suggest that psoriasin in addition to other factors demonstrated by us as well as others aim to outcompete the negative consequences of high glucose. We hypothesize that these factors although partly acting independently may still be interrelated thereby strengthening the effect.

Taken together, our data suggest psoriasin as an important antimicrobial peptide in bacterial clearance of the urinary tract in diabetes and may in the future serve as a potential target for new therapeutic drugs.

**Methods**

**Study participants and collection of human serum, plasma and urine exfoliated cells**

The study was approved by the Regional Ethics Committee, Stockholm and performed in accordance with the Helsinki Declaration. Informed consent was obtained from all patients and volunteers participating in the study. Adult individuals with prediabetes (ethics permission 96:300), diabetes mellitus (DM) and non-diabetic controls (ethics permissions 2010/723-3/1 (amendment 2018/603-32), 2013/1618-31/3 (amendment 1500-32), 2008/1804-31 (amendment 2017/477-32)) were included. Patients and volunteers with ongoing UTI, any antimicrobial treatment or estrogen supplementation, were excluded. Venous blood glucose, HbA1c and urine creatinine were analysed in all participants and volunteers.

**Cell lines and culture conditions**

Telomerase-immortalized human uroepithelial cells, TERT-NHUC (kindly provided by M. A. Knowles, Leeds, UK) and 5637 (HTB-9, American Type Culture Collection) were cultured as previously described. TERT NHUC cells were grown in EpiLife medium (6 mM of glucose, Life Technologies) supplemented with 1% of human keratinocytes growth supplement (HKGS, Life technologies) and 5637 cells in RPMI 1640 medium with no glucose (Life Technologies) but supplemented with 5 mM glucose (Sigma) and 10% fetal bovine serum (Life Technologies) and cultured at 37°C and 5% CO₂. To mimic hyperglycemia, cells were exposed to 11 and 30 mM of glucose for 24 to 72 h as appropriate, while normoglycemia, 6 mM, was obtained with culture media for TERT-NHUC while 5637 cells were supplemented without cytotoxicity or compromising proliferations (Suppl. Fig. 1h, respectively). For experiments with estradiol, phenol red–free medium and 5% charcoal–treated fetal bovine serum were used. 17β-estradiol (Sigma) in absolute ethanol was used at a final concentration of 10 nM. Cells were treated for 48 h, with medium exchanged after 24 h. For experiments with L-NAME (Sigma), 5 μM of N,N,N′,N′-tetramethyl (2-pyridylmethyl) ethylenediamine, (TPEN; Sigma) was added to human uroepithelial cells, TERT-NHUC for 2 h in minimal essential medium (MEM; Life Technologies), followed by treatment with high glucose for a total 24 h in Epiline medium.

**Bacterial strain**

Uropathogenic *E. coli* strain CFT073 was used for in vitro and in vivo experiments. This strain was isolated from a patient with acute pyelonephritis and expresses type 1, P and S fimbriae along with α-hemolysin. Bacteria were grown over night on blood agar plates at 37°C followed by 4 h in Luria-Bertani broth to reach the logarithmic phase. Bacteria were washed twice with phosphate-buffered saline (PBS) and bacterial concentration was measured spectrophotometrically and confirmed by viable count.

**Mouse model of UTI**

Mice experiments were approved by the Northern Stockholm Animal Ethics Committee (animal ethics permission N-177/14 (amendment 10370-2018)), and experiments were carried out according to the guidelines of the Federation of Laboratory Animal Science Association and in compliance with the Committee’s requirements. Eight-week-old female db/db (BKS (D)-Leprd/b) mice with type 2 diabetes (median blood glucose: 17.1 mmol/l) and wildtype C57BL/6j mice (median blood glucose 7.1 mmol/l) were obtained from Janvier Laboratories. All mice were kept in a specific pathogen free facility in individual ventilated cages with aspen bedding housing 4 mice per cage. At 12 h light, 12 h dark cycle in ambient room temperature and humidity was maintained, with food and water ad libitum. At week 10, infection was performed. Water was withdrawn 4 h prior to bacterial inoculation, mice were anaesthetized using isoflurane and transurethrally infected with 0.5 × 10⁸ colony-forming units (CFU) of *E. coli* CFT073 in 50 μl of PBS, after which water was returned. Blood glucose levels for db/db and C57BL/6j were measured before infection, during the course of infection and at sacrifice after 24 h, 7 or 14 days of infection. Urine was collected and respective urinary bladders were aseptically removed, cut open and washed with PBS to remove urine and non-adherent bacteria. To determine the total bacterial load, adhered and intracellular bacteria, bladders were homogenized in 1 ml of PBS, serially diluted and both bladders and urine were plated on blood agar plates.

**Cell infection assays**

Cell experiments were carried out in 24 well cell culture plates, human uroepithelial cells TERT-NHUC in Primaria (BD) and 5637 cells in Costar plates. Cells were grown in the presence of 5 or 6, 11 and 30 mM...
Adhesion and survival assay
TERT-NHUC and 5637 cells were infected with \(10^6\) E. coli in 100 µl of PBS per well, centrifuged for 1 min at 350 g. For assessment of bacterial adhesion, cells were infected for 30 mins only and washed with PBS. In survival assays, cells were washed with PBS after 2 h infection to remove non-adherent bacteria and supplemented with fresh medium for another 2 h. In hyperglycemic cells, medium was supplemented with 30 mM glucose throughout the entire experiment. At indicated time points, cells were lysed with 0.1% Triton-X-100 in PBS (pH 7.4) and serially diluted and plated on blood agar plates. Rate of adhesion and survival were calculated by number of adhered or intracellular bacteria in relation to the total number from the same experiment.

Antimicrobial activity, effect of psoriasin
Human uroepithelial cells, TERT-NHUC were treated with 6 or 30 mM glucose. After 24 h of treatment, medium was removed, and cells were lysed in 1% Triton-X-100. Cell free supernatant was obtained by centrifugation at 8000 g for 5 mins and then incubated for 30 mins at \(37^\circ C\) with 1 µg/ml of monoclonal mouse anti-psoriasin antibody (Santacruz Biotechnology) or the same concentration of an isotype control antibody (mouse (Ig)G1, (Santacruz Biotechnology) or the same concentration of an isotype control antibody (mouse (Ig)G1, (Santacruz Biotechnology). Bacteria were prepared as above and 50 µl from \(10^4\) CFU/ml bacterial suspension was added to 150 µl of pretreated cell lysate. After incubation for 30 min at \(37^\circ C\), 100 µl aliquots were plated and bacterial survival was determined by viable count. Results were expressed in relation to control cell lysates pretreated with control antibodies.

Urine samples, with no bacterial growth from nondiabetic controls and T2D patients were collected, and E. coli CFT073 was prepared using the RNeasy Mini kit (Qiagen) according to the manufacturer’s protocol. The concentration and purity of RNA was determined with a nanodrop, and up to 0.5 µg of RNA was reverse transcribed using random primers for 10 min at 25 °C, 120 min at 37 °C, and inactivation at 85 °C for 5 min and was transcribed to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) in thermal cycler, MJ research, PTC-200. Real-time PCR was performed after initial denaturation at 95 °C for 10 min, each cycle consisted of 15 s at 95 °C, 60 s at 60 °C (touchdown of 1 °C per cycle from 66° to 60 °C), and 30 s at 72 °C using standard SYBR green (Applied Biosystems) or using probes in TaqMan gene expression assays (Applied Biosystems) for both human and mouse specific genes in a Rotor-Gene PCR cycler (Corbett Life Science, RG 3000, rotor gene version 6.1). All the primers and probes used in this study are mentioned in supplementary table 2. Relative expressions of target genes were presented as 2^ΔΔCT and fold change as 2^ΔCt compared to uninfected or non-treated control.

Immunofluorescence of bladder sections and cells
Mice experiments were performed as described earlier. At the required time point, bladder tissue was fixed in 1 ml of 4% PFA for at least 36 h, and then transferred to 1 ml of absolute ethanol. Paraffin blocks were prepared and 4 µm sections were cut using microtome. Sections of paraffin-embedded mouse bladder tissue were deparaffinized and rehydrated, pretreated with 0.3% Triton X-100/PBS at room temperature, or boiled in citrate buffer, 1 mM EDTA, 10 mM tris, 0.05% Tween 20 (pH 9); for psoriasin (S100a7a), IL-1β, IL-6, mannos receptor c-type 1 (Mrcl; CD206), caveolin 1 (Cavl), occludin (Ocln) and RhoB staining. TERT-NHUC, human uroepithelial cells were fixed in 4% PFA for 15 mins at room temperature and permeabilized with 0.3% Triton X-100 in PBS. Thereafter, sections were blocked for 30 mins with FX Signal Enhancer (Invitrogen), and both cells and sections were blocked for an additional 60 mins with the sera from the species in which the secondary antibodies were raised. Incubation with primary antibodies was carried out overnight at 4 °C followed by secondary antibodies for 1 h at room temperature. Antibodies used were goat anti E. coli (1:200, AbD Serotec; chicken anti goat Alexa Fluor 594 or 488 (1:600), Invitrogen), rabbit anti uropakin Ila (UPilha, 1:200, Santacruz; donkey anti rabbit Alexa Fluor 488 (1:600), Invitrogen), mouse anti psoriasin (1:200, Santacruz; rabbit anti mouse Alexa Fluor 488 (1:600), Invitrogen), mouse anti occludin (tissue: 1:200, Santacruz; rabbit anti mouse Alexa Fluor 594 (1:600), Invitrogen) or rabbit anti occludin (cells: 1:200, Invitrogen; donkey anti rabbit Alexa Fluor 488 (1:600), Invitrogen), mouse anti caveolin 1 (1:200, Santacruz; rabbit anti mouse Alexa Fluor 594 (1:600), Invitrogen), mouse anti mannos receptor c-type 1 (CD206, 1:100, Abcam; donkey anti rabbit Alexa Fluor 488 (1:600), Invitrogen), rabbit anti IL-1β (1:200, Invitrogen; donkey anti rabbit Alexa Fluor 488 (1:600), Invitrogen), rabbit anti IL-6 (1:100, Invitrogen; donkey anti rabbit Alexa Fluor 594 (1:600), Invitrogen), TRITC-labeled phalloidin (1:350, Sigma), Phalloidin Alexa Fluor 488 (1:1000; Invitrogen), rabbit anti RhoB (1:200, Invitrogen; donkey anti rabbit Alexa Fluor 485 or 594 (1:600), Invitrogen) and YAP/TAZ (1:100, Santacruz; rabbit anti mouse Alexa Fluor 488 (1:500), Invitrogen). Sections and cells were then mounted with ProLong Gold Antifade mounting medium including DAPI (Invitrogen). For nuclear YAP/TAZ estimation, the integrated density module in Imagej was used to measure the total fluorescence intensity of each cell, followed by the fluorescence intensity in the nucleus of each cell. The proportion of nuclear over total cellular YAP/TAZ was calculated. Analysis of the type of actin filament organization was done by analyzing images acquired by immunofluorescence microscopy for the presence of short actin bundles, stress fibers or cortical actin as the dominant form of actin organization. Quantification was performed from three independent experiments per experimental condition. Imaging was performed with Leica SP5, Zeiss LSM 700 confocal microscopes and Zeiss AxioVert 40 CFL epifluorescence microscope, fluorescence intensity per unit area were analyzed in image J Fiji 1.53b software.

Psoriasis, occludin and IL-6 ELISA
Serum and plasma from patients with diabetes, along with hyperglycemic clamped pre-diabetic and hyperinsulinemia clamped TID and non-diabetic control samples were collected before and after 2 h of glucose or insulin treatment respectively. Supernatants from 24 h glucose treated TERT-NHUC cells were collected and centrifuged at 350 g for 10 min and stored at –80 °C until assayed. ELISA were analyzed using the CircuLex S100A7/Psoriasin ELISA Kit (MBL International) and IL-6 (R&D Biosystems) according to the manufacturer’s recommendations in EZ400 microplate reader (Biochrom) using ADAP software version 2.0. Uninfected cells and serum and plasma obtained from nondiabetic individuals and before glucose and insulin infusion served as controls.

Flow cytometry
To investigate the protein expression of psoriasin, occludin, and caveolin 1, TERT-NHUC cells were harvested after 36 h glucose treatment. For MRCl (CD206), cells were further infected for 2 h, centrifuged at 350 g for 3 min at room temperature (RT). 1 ml of 4% PFA in PBS (Fisher Scientific) was added to the cell pellet. Cells were
incubated at RT for 15 mins, centrifuged and 1 ml of 0.1\% Triton-X-100 in PBS (PBST) was added and incubated in RT for 10 min. Thereafter cells were blocked with 5\% BSA for 30 min, stained with primary antibody in 1:1 ratio of 200 \\( \mu \)L of 1:1 PBST and 5\% BSA for 30 mins at RT. After primary antibody staining, cells were washed with 1:1 PBST with 1\% BSA and further incubated with respective secondary Alexa fluor 488 (1:600, Invitrogen) or Alexa fluor 647 (1:400, Invitrogen) antibodies in 1:1 ratio of 200 \\( \mu \)L of 1:1 PBST and 5\% BSA for 25 min in dark at RT.

Antibodies used are rabbit anti pSTAT-3 (1:100, Cell Signaling Technology; donkey anti rabbit Alexa Fluor 488 (1:400, Invitrogen), mouse anti STAT 3 (1:100, Cell Signaling Technology; goat anti mouse Alexa Fluor 647 (1:400), Invitrogen), mouse anti psoriasin (1:200, Santa Cruz; rabbit anti mouse Alexa Fluor 488 (1:400), Invitrogen), mouse anti cavelin I (1:200, Santacruz; rabbit anti mouse Alexa Fluor 488 (1:400), Invitrogen), and rabbit anti mannose receptor c-type 1 (1:200, Invitrogen; donkey anti rabbit Alexa Fluor 488 (1:400), Invitrogen), mouse anti S100A7 (1:600, Invitrogen), and negative cell populations. Representative contour plot for each protein of interest are shown in Supplementary Fig. 5.

S100A7 deletion using crispr/cas9 system

Human uroepithelial cells TERT-NHUC were cultured in 24-well plates to 50–70\% confluency and transacted with non-targeting SpCas9/gRNA (Synthego) or a pool of S100A7 specific SpCas9/gRNA (Synthego) using Lipofectamine\textsuperscript{TM} CRISPRMAX\textsuperscript{TM} Cas9 Transfection Reagent (Thermo Scientific) according to the instructions of the manufacturer. After transfection the TERT-NHUC cells were cultured for 72 h and then fixed and processed for microscopy analysis of mouse anti psoriasin (1:200, Santacruz; rabbit anti mouse Alexa Fluor 488 (1:600), Invitrogen) and rabbit anti occludin (1:200, Invitrogen; goat anti rabbit Alexa Fluor 647 (1:400), Invitrogen). Finally, cells were dissolved in PBS and data acquired on a BD LSFortessa\textsuperscript{TM} and analyzed in FlowJo software version 10.8.1. The gating strategy used standard FSC and SSC, indicating boundaries between positive and negative cell populations. Representative contour plot for each protein of interest are shown in Supplementary Fig. 5.

RHOA/B transfection

Human uroepithelial cells 5637 were cultured in 24 well plate. When 70\% confluency was reached, cells were pretreated with high glucose for at least 6 h prior to the transfection. 1 \\( \mu \)g of Myc-tagged RHOA/T19N and RHOB/T19N were separately prepared in 150 mM NaCl to a final volume of 50 \\( \mu \)L. 2 \\( \mu \)L of jetPEI\textsuperscript{TM} reagent in 150 mM NaCl to a final volume of 50 \\( \mu \)L. 50 \\( \mu \)L jetPEI\textsuperscript{TM} solution was added all at once into the 50 \\( \mu \)L DNA solution and incubated for 20 min at room temperature. 100 \\( \mu \)L jetPEI\textsuperscript{TM}/DNA mix was added drop wise to the cells in 1 mL of serum-containing medium and homogenized by gently swirling the plate. Cells were incubated for 24 h followed by fixation with 1 mL of 4\% PFA and were processed for microscopy analysis of mouse anti YAP/TAZ (1:100, Santa Cruz) detected by rabbit anti mouse Alexa Fluor 488 (1:500, Invitrogen) conjugated antibody and Myc-tagged RHOA/T19N and RHOB/T19N detected with rabbit anti Myc antibody (1:200, Sigma Aldrich) followed by donkey anti rabbit Alexa Fluor 350 (1:500, Invitrogen) conjugated antibody using standard imaging protocol.

Peptides

Purified Zn\textsuperscript{2+} free, natural skin derived, 11,366-Da psoriasin peptide\textsuperscript{15} and recombinant human IL-1\( \beta \) (Milkenyi Biotec) and IL-6 (Invitrogen) were used to stimulate TERT-NHUC for 24 h. The concentrations used were 1600 nM or 5 \( \mu \)M, 20 ng/ml and 50 ng/ml respectively. IL-1\( \beta \) blocking was achieved by addition of IL-1\( \beta \) specific inhibitor, diacerein (Sigma, 50 \( \mu \)M) for at least 4 h prior to the IL-1\( \beta \) peptide treatment followed by overnight treatment in high glucose treated TERT-NHUC.

Statistical analysis

All statistical tests were performed in Graph pad Prism version 5. No samples were excluded from human and animal studies. For in vitro analysis using human uroepithelial cells, statistical outliers defined by Grubb’s test were excluded. Data were obtained from Students unpaired t-test, non-parametric test using Mann Whitney U test, paired Students t-test and non-parametric one-way ANOVA, with Bonferroni’s or Dunnett’s multiple comparison tests as appropriate. Differences with p values below 0.05 were considered statistically significant.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Our data do not mandate deposition in a public repository. All raw files and other relevant information are stored in the Karolinska Institutet’s Electronic Lab Notebook. As the Karolinska Institutet’s Electronic Lab Notebook is not a public repository, information may be provided from the corresponding author upon reasonable request. Source data are provided with this paper as a Source Data file.

References

1. Maffi, P. & Secchi, A. The burden of diabetes: emerging data. Dev. Ophthalmol. 60, 1–5 (2017).
2. Muller, L. M. et al. Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. Clin. Infect. Dis. 41, 281–288 (2005).
3. Geerlings, S. E. Urinary tract infections in patients with diabetes mellitus: epidemiology, pathogenesis and treatment. Int J. Anti. microb. Agents 31, S54–S57 (2008).
4. Zasloff, M. Why are diabetics prone to kidney infections? J. Clin. Invest. 128, S213–S215 (2018).
5. Nitzan, O., Elias, M., Chazan, B. & Saliba, W. Urinary tract infections in patients with type 2 diabetes mellitus: review of prevalence, diagnosis, and management. Diabetes Metab. Syndr. Obes. 8, 129–136 (2015).
6. Truzzi, J. C., Almeida, F. M., Nunes, E. C. & Sadi, M. V. Residual urinary volume and urinary tract infection when are they linked? J. Urol. 180, 182–185 (2008).
7. Becknell, B., Schwaderer, A., Hains, D. S. & Spencer, J. D. Amplifying renal immunity: the role of antimicrobial peptides in pyelonephritis. Nat. Rev. Nephrol. 11, 642–655 (2015).
8. Zasloff, M. Antimicrobial peptides, innate immunity, and the normally sterile urinary tract. J. Am. Soc. Nephrol. 18, 2810–2816 (2007).
9. Burgey, C., Kern, W. V., Romer, W., Sakinc, T. & Rieg, S. The innate defense antimicrobial peptides hBD3 and RNase7 are induced in human umbilical vein endothelial cells by classical inflammatory cytokines but not Th17 cytokines. Microbes Infect. 17, 353–359 (2015).
10. Mansour, S. C., Pena, O. M. & Hancock, R. E. Host defense peptides: front-line immunomodulators. Trends Immunol. 35, 443–450 (2014).
11. Becknell, B. et al. Expression and antimicrobial function of beta-defensin 1 in the lower urinary tract. PLoS ONE 8, e77714 (2013).
12. Chromek, M. et al. The antimicrobial peptide cathelicidin protects the urinary tract against invasive bacterial infection. Nat. Med. 12, 636–641 (2006).
13. Spencer, J. D. et al. Ribonuclease 7, an antimicrobial peptide upregulated during infection, contributes to microbial defense of the human urinary tract. Kidney Int. 83, 615–625 (2013).
14. Ostergaard, M., Wolf, H., Omhoff, T. F. & Celis, J. E. Psoriasin (S100A7): a putative urinary marker for the follow-up of patients
with bladder squamous cell carcinomas. *Electrophoresis* **20**, 349–354 (1999).

15. Glaser, R. et al. Antimicrobial psoriasin (S100A7) protects human skin from *Escherichia coli* infection. *Nat. Immunol.* **6**, 57–64 (2005).

16. Brauner, H. et al. Type 2 diabetes mellitus and the effect of the antioxidant coenzyme Q10 on inflammatory markers of innate immune activity in patients with type 1 and matury activity. *Clin. Exp. Immunol.* **177**, 478–482 (2014).

17. Eichler, T. E. et al. Insulin and the phosphatidylinositol 3-kinase signaling pathway regulate RiboNuclease 7 expression in the human urinary tract. *Kidney Int.* **90**, 568–579 (2016).

18. Wagner, H., Alvarsson, M., Mannheimer, B., Degerblad, M. & Ostenson, C. G. No effect of high-dose vitamin D treatment on beta-cell function, insulin sensitivity, or glucose homeostasis in subjects with abnormal glucose tolerance: a randomized clinical trial. *Diabetes Care* **39**, 345–352 (2016).

19. Rathmans, B., Rosfors, S., SJöholm, A. & Nyström, T. Early signs of atherosclerosis are associated with insulin resistance in non-obese adolescent and young adults with type 1 diabetes. *Cardiovasc. Diabetol.* **11**, 145 (2012).

20. Kolls, J. K., McCray, P. B. Jr. & Chan, Y. R. Cytokine-mediated regulation of antimicrobial proteins. *Nat. Rev. Immunol.* **8**, 829–835 (2008).

21. Han, H. et al. Loss of aryl hydrocarbon receptor suppresses the response of colonic epithelial cells to IL-22 signaling by upregulating SOCS3. *Am. J. Physiol. Gastrointest. Liver Physiol.* **322**, G93–g106 (2022).

22. Dabir, P., Marinic, T. E., Krivokovets, I. & Stenina, O. I. Aryl hydrocarbon receptor is activated by glucose and regulates the thrombospordin-1 gene promoter in endothelial cells. *Circ. Res.* **102**, 1558–1565 (2008).

23. Son, E. D. et al. S100A7 (psoriasin) inhibits human epidermal differentiation by enhanced IL-6 secretion through IkappaB/NF-kappaB signalling. *Exp. Dermatol.* **25**, 636–641 (2016).

24. West, N. R. & Watson, P. H. S100A7 (psoriasin) is induced by the proinflammatory cytokine oncostatin-M and interleukin-6 in human breast cancer. *Oncogene* **29**, 2083–2092 (2010).

25. Robinson, K., Deng, Z., Hou, Y. & Zhang, G. Regulation of the intestinal barrier function by host defense peptides. *Front Vet. Sci.* **2**, 57 (2015).

26. Hein, K. Z. et al. Disulphide-reduced psoriasin is a human apoptosis-inducing broad-spectrum fungicide. *Proc. Natl Acad. Sci. USA* **112**, 13039–13044 (2015).

27. Miyoshi, Y., Tanabe, S. & Suzuki, T. Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression. *Am. J. Physiol. Gastrointest. Liver Physiol.* **311**, G105–G116 (2016).

28. Ofek, I., Mirelman, D. & Sharon, N. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. *Nature* **265**, 623–625 (1977).

29. Saas, D. W., Swan, Z., Brown, B. J., Wright, J. R. & Abraham, S. N. The expanding roles of caveolin proteins in microbial pathogenesis. *Commun. Integr. Biol.* **2**, 535–537 (2009).

30. Hall, A. Rho GTPases and the actin cytoskeleton. *Science* **279**, 509–514 (1998).

31. Grande-García, A. et al. Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases. *J. Cell Biol.* **177**, 683–694 (2007).

32. Kong, F. et al. The Characteristic of S100A7 Induction by the Hippo-YAP pathway in cervical and glossoharyngeal squamous cell carcinoma. *PLoS ONE* **11**, e0167080 (2016).

33. Calvet, H. M. & Yoshikawa, T. T. Infections in diabetes. *Infect. Dis. Clin. North Am.* **15**, 407–421 (2001). viii.

34. Murtha, M. J. et al. Insulin receptor signaling regulates renal collecting duct and intercalated cell antibacterial defenses. *J. Clin. Invest.* **128**, 5634–5646 (2018).

35. Vegfors, J., Petersson, S., Kovács, A., Polyak, K. & Enerbäck, C. The expression of Psoriasin (S100A7) and CD24 is linked and related to the differentiation of mammary epithelial cells. *PLoS ONE* **7**, e53119 (2012).

36. Froy, O., Hananel, A., Chapnik, N. & Madar, Z. Differential effect of insulin treatment on decreased levels of beta-defensins and Toll-like receptors in diabetic rats. *Mol. Immunol.* **44**, 796–802 (2007).

37. Hiratsuka, T., Nakazato, M., Date, Y., Mukae, H. & Matsukura, S. Nucleotide sequence and expression of rat beta-defensin-1: its significance in diabetic rodent models. *Nephron* **86**, 65–70 (2001).

38. Morrison, G., Klanowski, F., Davidson, D. & Dorin, J. Characterization of the mouse beta defensin 1, Defb1, mutant mouse model. *Infect. Immun.* **70**, 3053–3060 (2002).

39. Lan, C. C. et al. High-glucose environment inhibits p38MAPK signaling and reduces human beta-defensin-3 expression [corrected] in keratinocytes. *Mol. Med.* **17**, 771–779 (2011).

40. Montoya-Rosalves, A., Castro-Garcia, P., Torres-Juarez, F., Enciso-Moreno, J. A. & Rivas-Santiago, B. Glucose levels affect LL-37 expression in monocyte-derived macrophages altering the Mycobacterium tuberculosis intracellular growth control. *Micro. Pathog.* **47**, 148–153 (2016).

41. Harder, J. & Schroder, J. M. RNase 7, a novel innate immune defense antimicrobial protein of healthy human skin. *J. Biol. Chem.* **277**, 46779–46784 (2002).

42. Schroeder, B. O. et al. Reduction of disulfide bonds unmasks potent antimicrobial activity of human beta-defensin 1. *Nature* **469**, 419–423 (2011).

43. Higgins, G. et al. Lipoxin A4 prevents tight junction disruption and delays the colonization of cystic fibrosis bronchial epithelial cells by *Pseudomonas aeruginosa*. *Am. J. Physiol. Lung Cell Mol. Physiol.* **310**, L1053–L1061 (2016).

44. Khandelwal, F., Abraham, S. N. & Apodaca, G. Cell biology and physiology of the uroepithelium. *Am. J. Physiol. Ren. Physiol.* **297**, F1477–F1501 (2009).

45. Montalbetti, N. et al. Urothelial tight junction barrier dysfunction sensitizes bladder afferents. *eNeuro* **4**, (2017).

46. Spoerri, P. E. et al. Effects of VEGFR-1, VEGFR-2, and IGF-IR hamster ribozymes on glucose-mediated tight junction expression in cultured human retinal endothelial cells. *Mol. Vis.* **12**, 32–42 (2006).

47. Bhattacharjee, P. S. et al. High-glucose-induced endothelial cell injury is inhibited by a Peptide derived from human apolipoprotein E. *PLoS ONE* **7**, e52152 (2012).

48. Hattori, F. et al. The antimicrobial protein S100A7/psoriasin enhances the expression of keratinocyte differentiation markers and strengthens the skin’s tight junction barrier. *Br. J. Dermatol.* **171**, 742–753 (2014).

49. Akiyama, T. et al. The human cathelicidin LL-37 host defense peptide upregulates tight junction-related proteins and increases human epidermal keratinocyte barrier function. *J. Innate Immun.* **6**, 739–753 (2014).

50. Kiatsurayanon, C. et al. Host defense (Antimicrobial) peptide, human beta-defensin-3, improves the function of the epithelial tight-junction barrier in human keratinocytes. *J. Invest. Dermatol.* **134**, 2163–2173 (2014).

51. Ozer, A. et al. Advanced glycation end products facilitate bacterial adherence in urinary tract infection in diabetic mice. *Pathog Dis.* **73**, (2015).

52. Moreno-Vicente, R. et al. Caveolin-1 modulates mechanotransduction responses to substrate stiffness through actin-dependent control of YAP. *Cell Rep.* **25**, 1622–1635 e1626 (2018).
and the human objects in Fig. 1c, d. These figures were made using a paid subscription with BioRender.

Author contributions

S.M. and A.B. conceived and designed the experiments; S.M., W.K., A.S., P.A. performed research; A.B.j., J.T., K.B., T.N., C.-G.Ö., H.B. contributed human samples; A.E., J.S., C.-G.Ö., H.B., A.B. provided reagents/new analytic tools; S.M., W.K., A.S., C.-G.Ö., P.A., H.B. and A.B. analyzed data; S.M. and A.B. wrote the paper; A.B. supervised the work. All authors read and approved the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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Veranic, P., Romih, R. & Jezernik, K. What determines differentiation of urothelial umbrella cells? Eur. J. Cell Biol. 83, 27–34 (2004).

Eto, D. S., Sundsökab, J. L. & Mulvey, M. A. Actin-gated intracellular growth and resurgence of uropathogenic Escherichia coli. Cell Microbiol. 8, 704–717 (2006).

Ostenson, C. G., Gaisano, H., Sheu, L., Tibell, A. & Bartfai, T. Impaired gene and protein expression of exocytotic soluble N-ethylmaleimide attachment protein receptor complex proteins in pancreatic islets of type 2 diabetic patients. Diabetes 55, 435–440 (2006).

Lv, Z. et al. Fyn mediates high glucose-induced actin cytoskeleton reorganization of podocytes via promoting ROCK activation in vitro. J. Diabetes Res. 2016, 5671803 (2016).

Halder, G., Dupont, S. & Piccolo, S. Transduction of mechanical and cytoskeletal cues by YAP and TAZ. Nat. Rev. Mol. Cell Biol. 13, 591–600 (2012).

Qi, Z. et al. The characteristics and function of S100A7 induction in squamous cell carcinoma: heterogeneity, promotion of cell proliferation and suppression of differentiation. PLoS ONE 10, e0128887 (2015).

Spiering, D. & Hodgson, L. Dynamics of the Rho-family small GTPases in actin regulation and motility. Cell Adh Migr. 5, 170–180 (2011).

Moorby, S., Byfield, F. J., Janmey, P. A. & Klein, E. A. Matrix stiffness regulates endosomal escape of uropathogenic E. coli. Cell Microbiol. 22, e13196 (2020).

Mysorekar, I. U. & Hultgren, S. J. Mechanisms of uropathogenic Escherichia coli persistence and eradication from the urinary tract. Proc. Natl Acad. Sci. USA 103, 14170–14175 (2006).

Ozer, A. et al. Impaired cytokine expression, neutrophil infiltration and bacterial clearance in response to urinary tract infection in diabetic mice. Pathog Dis 73, (2015).

Handgraaf, S., Dusaülçü, R., Visentin, F., Philippe, J. & Gosmain Y. 17-beta Estradiol regulates proglucagon-derived peptide secretion in mouse and human alpha- and L cells. JCI Insight 3, (2018).

Yan, H. et al. Estrogen improves insulin sensitivity and suppresses gluconeogenesis via the transcription factor Foxo1. Diabetes 68, 291–304 (2019).

Brit, V. & Baudry, M. Estrogen regulates protein synthesis and actin polymerization in hippocampal neurons through different molecular mechanisms. Front Endocrinol. (Lausanne) 5, 22 (2014).

Skliris, G. P. et al. Estrogen receptor-beta regulates psoriasin (S100A7) in human breast cancer. Breast Cancer Res. Treat. 104, 75–85 (2007).

Mohanthy, S. et al. HIF-1 mediated activation of antimicrobial peptide LL-37 in type 2 diabetic patients. J. Mol. Med. (Berl.) 100, 101–113 (2022).

Tabák, A. G., Herder, C., Rathmann, W., Brunner, E. J. & Kivimäki, M. Prediabetes: a high-risk state for diabetes development. Lancet 379, 2279–2290 (2012).

Hertting, G., Lutjhe, P., Sullivan, D., Aspenstrom, P. & Brauner, A. Vitamin D-deficient mice have more invasive urinary tract infection. PLoS ONE 12, e0180810 (2017).

Lutjhe, P. et al. Estrogen supports urothelial defense mechanisms. Sci. Transl. Med. 5, 190ra180 (2013).