# Mini Lectures

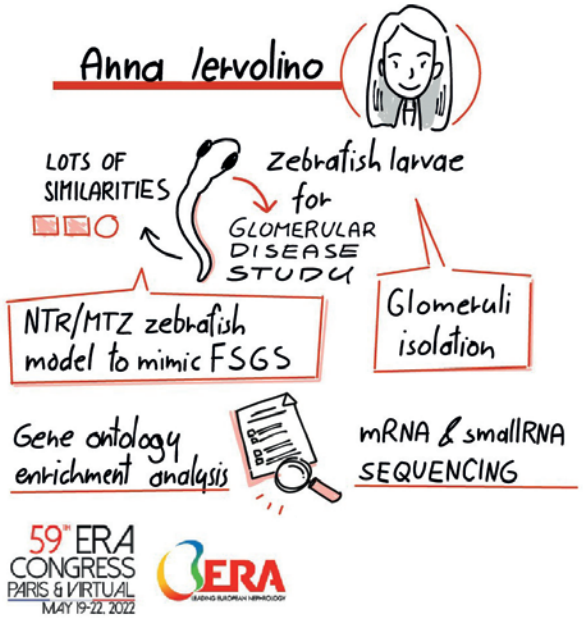
## Zebrafish larvae to study and treat glomerular diseases

Anna Iervolino, Italy

The zebrafish pronephros is a well-established model to study glomerular development, structure, and function since it contains the same cell types as humans and mice. Furthermore, the zebrafish larvae are optically transparent, allowing researchers to observe the processes in real-time. The filtration in the glomerulus begins two days post-fertilization, making this model highly relevant for the investigation of human glomerular kidney diseases. Several transgenic zebrafish models have been developed in the recent decade to examine glomerular disease by visualizing the podocyte body, monitoring the primary foot processes, and investigating renal progenitor cells. By using the Morpholino system to knock down specific podocyte markers, researchers discovered ultrastructural glomerular damage, proteinuria, edema, increased embryonic mortality, and deformities.

Focal segmental glomerulosclerosis (FSGS) was the focus of the research of the group from the University of Medicine in Greifswald. Although causative factors, such as mutations in key podocyte genes have already been identified, the pathogenic cause of the majority of FSGS cases is unknown. The group used a nitroreductase/metronidazole (NTR/MTZ) transgenic zebrafish model to achieve podocyte ablation and mimic FSGS. MTZ-treated larvae developed edema as a hallmark of the glomerular filtration barrier damage and severe proteinuria occurred due to podocyte apoptosis and progressive effacement of the foot process. Additionally, activation of proximal tubule-like parietal epithelial cells identified by ultrastructural cytomorphology, and expression of proximal tubule markers were observed. The glomeruli were then isolated from MTZ-treated zebrafish larvae using the fluorescence microscope to perform mRNA and miRNA sequencing. Gene ontology enrichment analysis revealed an up-regulation of metabolic processes, immune response, and ion transport and down-regulation of nephron development and slit membrane-associated proteins.

In many important aspects, the glomerular response to podocyte reduction in larval zebrafish is comparable to that of human FSGS. A thorough understanding of these mRNA and miRNA-based gene regulatory mechanisms will aid in the discovery of the pathomechanism and the development of therapeutics for the treatment of FSGS.



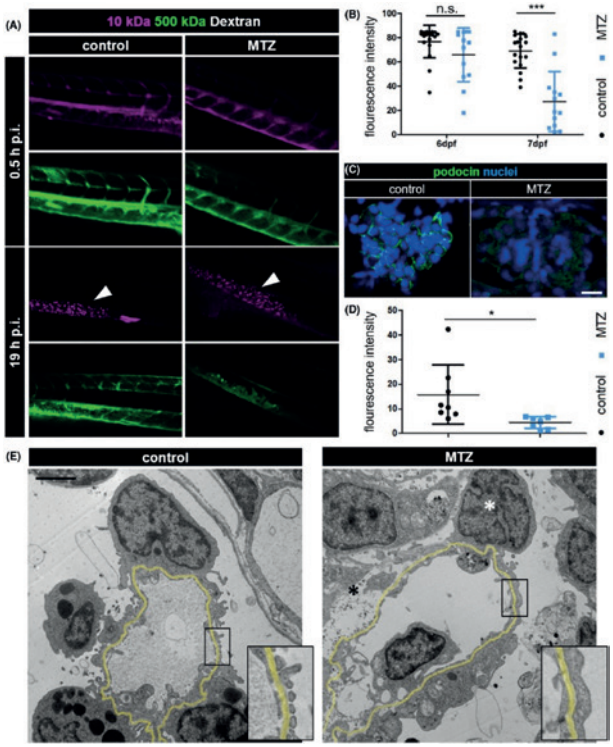




**Figure 1.**
Metronidazole induces podocyte injury and progressive effacement of foot processes (from ref. 3)

## The role of SGLT2 inhibition in heart failure: What can nephrologists learn from cardiologists?

Nikolaus Marx, Germany

Current guidelines by the European Society of Cardiology recognize three types of heart failure: heart failure with preserved ejection fraction (HFpEF), heart failure with mildly reduced ejection fraction (HFmrEF), and heart failure with reduced ejection fraction (HFrEF). All types exhibit high morbidity and mortality and there is a clear need for upgrading current treatment strategies. The prognosis is even worse in patients with HFrEF and CKD.

The DAPA-HF trial was the first to assess the effect of the sodium-glucose cotransporter-2 (SGLT2) inhibitor dapagliflozin in patients with HFrEF. It concluded that SGLT2 inhibition significantly reduces cardiovascular death or worsening of heart failure in this population irrespective of diabetes. The EMPEROR-reduced trial showed similar results for empagliflozin. Meta-analysis of these trials showed a very robust and significant reduction of the combined risk of cardiovascular death or hospitalization for heart failure. The results were consistent in patients with and without chronic kidney disease (CKD) and even suggested that these agents also improve renal outcomes. HFpEF and HFmrEF patients were the focus of the EMPEROR-preserved trial which investigated the safety and efficacy of empagliflozin. It showed a highly significant and robust risk reduction of cardiovascular death or hospitalization for heart failure in these patient cohorts, regardless of the presence or absence of diabetes. Looking at kidney function, the results were consistent both in patients with preserved kidney function as well as in those with eGFR ≤60mL/min. Finally, the DAPA-CKD trial found that dapagliflozin reduced the risk of kidney failure, cardiovascular death or hospitalization for heart failure, and overall mortality in patients with CKD, with and without type 2 diabetes. Therefore, SGLT2 inhibitors are associated with a convincing reduction of heart failure-related events in patients with CKD, patients with HFrEF, and patients with HFpEF.

Various mechanisms seem to contribute to the beneficial effects of SGLT2 inhibitors. EMPA Hemodynamics study in patients with type 2 diabetes and high cardiovascular risk looked at echocardiographic parameters of cardiac function as a secondary endpoint and showed that empagliflozin significantly improved diastolic function. Research suggests that, in comparison to loop diuretics, SGLT2 inhibitors may selectively inter-reduce interstitial fluid, which may limit the reflex neurohumoral stimulation that usually occurs in response to intravascular volume contraction with traditional diuretics. Mediation analysis from the EMPA-REG outcome trial suggested that the increase in hematocrit and hemoglobin may contribute to the overall result, and the EMPA Hemodynamics showed that empagliflozin leads to an increase in hemoglobin and hematocrit. Finally, it has been suggested that metabolic effects may play a role in this context: if an SGLT2 inhibitor is introduced, glucose levels decrease and other energy sources, such as ketones and branched-chain amino acids, may come into metabolic play again.

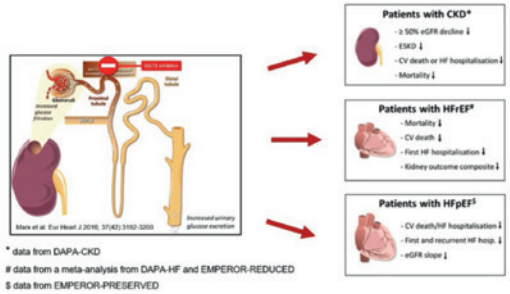



**Figure 2.**
The mechanism of action of SGLT2 inhibitors

## Food as medicine – can it link the gut microbiota to an improved renal phenotype?

Peter Stenvinkel, Sweden

Foodome is a new discipline that studies the food and nutrition domains through the application and integration of advanced omics technologies to improve well-being, health, and knowledge, and it includes a pool of all the compounds that make up the human diet. Current research in this domain focuses on linking individual eating patterns to the individual genome and the individual diseases and providing a tailor-made composition of nutrients that would have a favorable impact on health. A landmark study published in 2019 showed that a suboptimal diet is responsible for more deaths than any other traditional risk factor, including tobacco smoking, and fueled interest in the potential of the concept of food as medicine. Further works suggested that food could be used as a novel strategy to target the uraemic phenotype, characterized by epigenetic alterations, gut dysbiosis, mitochondrial dysfunction, inflammation, oxidative stress, and premature aging. One of the approaches would be targeting transcription factors involved in inflammation and oxidative stress, such as sulforaphane in broccoli, curcumin in turmeric,

anthocyanins in berries, etc. Another opportunity is to target senescent cells which have a pro-inflammatory effect by using nutrients that act as as senolytics and lead to apoptosis. Using the quercetin from apples, curcumin from turmeric, fisetin from strawberries, and epigallocatechin from green tea, it may be possible to down-regulate the senescence-associated secretory phenotype (SASP) response.

The major opportunity of using food as medicine is to target gut dysbiosis, which is promoted by the metabolic alterations of uremia and associated with inflammation and increased cardiovascular risk. Nutrients like fibers, prebiotics found in soybeans and wheat, polyphenols in grapes, coffee, and berries, as well as urolithin from berries and pomegranate, could alter the diversity of the gut microbiota. Research into the footprint of the systemic microbial biodiversity in advanced CKD shows that the decline of renal function is accompanied by altered microbial biodiversity. Gut dysbiosis predict increased mortality in dialysis patients. The links between gut microbiota and CKD are multifold, and research shows that one of the most important links is Trimethylamine N-oxide (TMAO). TMAO promotes pro-thrombotic effects and is associated with all-cause mortality. Preliminary data indicate that a diet rich in red meat and low in fish may increase the risk of death and shorten the time to initiation of renal replacement therapy. The generalized application of food as medicine should be based on solid scientific support. Thus, more studies are needed to prove the exact links, in order to utilize bioactive nutrients that benefit health and/or counteract the negative effects of drug treatment on the gut microbiota.

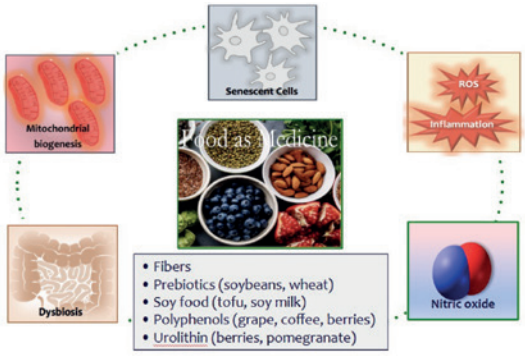
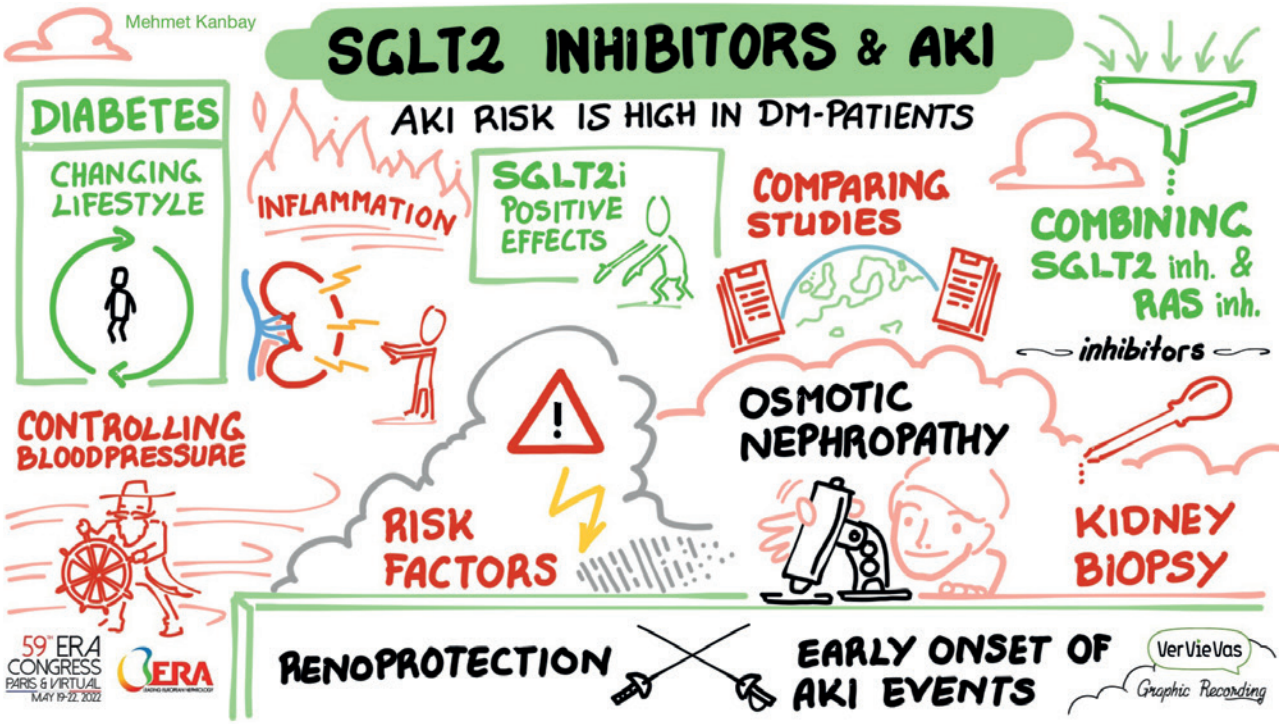



**Figure 3.**
Foods to target gut dysbiosis

## Sodium-Glucose Cotransporter-2 Inhibitors (SGLT2i) & Acute Kidney Injury (AKI)

Mehmet Kanbay, Turkey



SGLT2 inhibitors showed compelling improvement in the outcome of patients with diabetic kidney disease by hindering the progression of albuminuria and eGFR decline, in addition to blood glucose regulation, blood pressure control, and body weight management. In the light of the strong evidence presented in the CREDENCE and DAPA-CKD trials, KDIGO 2020 Clinical Practice Guidelines for Diabetes Management in CKD recommend the use of SGLT2i as part of first-line therapy for patients with eGFR ≥30mL/min. Nevertheless, renin-angiotensin system inhibitors or angiotensin receptor blockers (ARBs) should remain the first-line therapy for diabetic patients as they reduce albuminuria, the decline in filtration rate, fibrosis, inflammation and regulate hypertension.

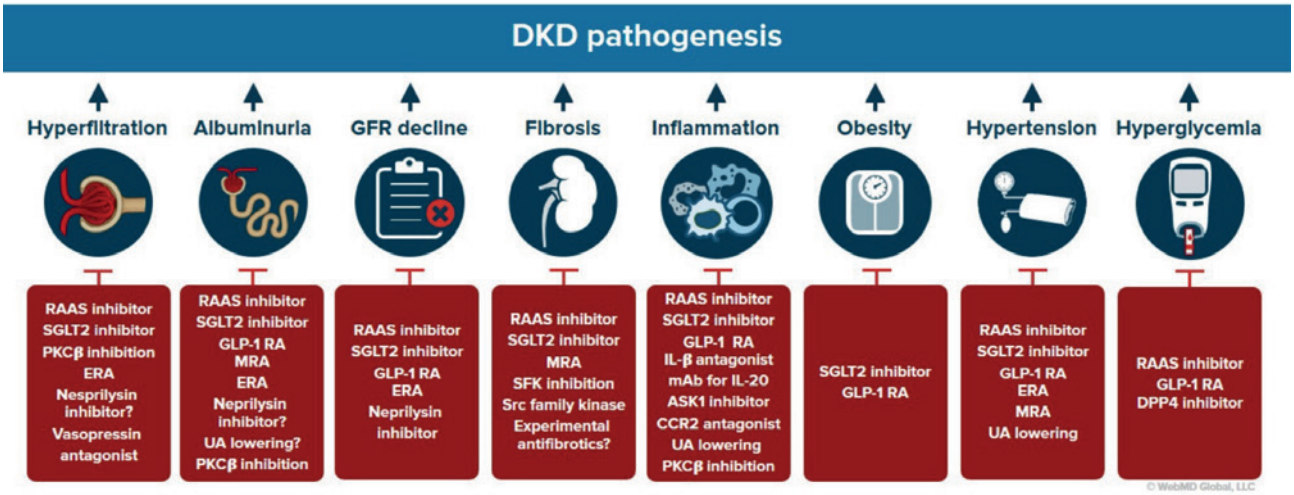



**Figure 4.**
Mechanisms of renoprotection with glucose-lowering therapies (from ref. 12)

However, despite the well-documented long-term renoprotective effects of SGLT2 inhibitors, postmarketing reports suggested an early onset of acute kidney injury (AKI) events, particularly with canagliflozin and dapagliflozin. These data have recently been questioned, since the research did not include a specified control group, and a propensity analysis suggested that SGLT2i carry an overall reduced AKI risk, compared to patients with type 2 diabetes with similar clinical characteristics. Some cases of AKI might relate to the acute reduction in eGFR that occurs with the initiation of SGLT2i therapy, likely due to the hemodynamic effect of the drugs to reduce glomerular pressure, and others may be caused by volume depletion triggered by the use of diuretics and angiotensin-converting enzyme (ACE) inhibitors or ARBs that augment the salt and water loss associated with SGLT2-dependent osmotic diuresis.

Many studies were initiated to explore whether SGLT2 inhibitors were related to the development of AKI. Dekkers et al. study looked at the effects of dapagliflozin on glomerular and tubular injury markers and showed that dapagliflozin significantly decreased kidney injury molecule 1 (KIM1) and interleukin 6 levels, pointing at beneficial effects on renal inflammation and reduction in proximal tubular cell injury. No change in global damage markers was observed. Katsuhara et al. studied the correlation between SGLT2i and acute renal failure in patient groups from Japan and outside of Japan. In non-Japanese patients with diabetes, there was a correlation between SGLT2 inhibitors use and the onset of acute renal failure that was not seen in cases reported in Japan. Furthermore, this study indicated that the signal of acute renal failure tended to be reduced in cases with the concomitant use of either an ACEi or ARB. Real World Data retrospective propensity match study from Canada compared SGLT2i and other glucose-lowering drugs concluding that SGLT2i use was not significantly associated with a higher risk of AKI compared to other drugs.

The mechanisms of reducing the AKI incidence with SGLT2 inhibitor use in type 2 diabetes are unclear. Some potential factors contributing to SGLT2 inhibitors-associated renoprotection may include decreasing oxygen and energy requirements of the tissue, diminished signals promoting tubular growth, reduced ischemia-reperfusion injury, and AKI, cardiorenal protection due to immunomodulatory effects, reduced inflammation and preservation of tubular integrity.

## Further readings

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*All the speakers reviewed and approved the content.*