Sir,

With great interest we read the article by Chang et al. [1] on the enhancement of epithelial sodium channel mRNA expression and protein level in renal cortical collecting duct (CCD) cells by advanced glycation end products (AGEs). The authors show that AGEs can increase sodium uptake in CCD cells in a dose-dependent way, providing a novel mechanism for the dysregulation of sodium balance in diabetic nephropathy.

However, we have concerns on their in vitro preparation of AGEs. Since their results are dependent on the stimulation of renal CCD cells with AGEs prepared in vitro, it is very important that they prepared their AGEs properly. For instance, Valencia et al. [2] showed that AGE preparations which were free of significant levels of endotoxin contamination, failed to induce proinflammatory cellular responses, whereas endotoxin induced cell surface VCAM-1 on HMEC-4 endothelial cells and tumour necrosis factor-α secretion by primary human PBMCs. Therefore, to exclude the possibility that the effects of AGEs on epithelial sodium channel expression in renal CCD are mediated by endotoxin contamination, the authors should have used endotoxin-free bovine serum albumin (BSA) and assayed the control and AGE preparation for endotoxin contamination, by using, for example, the Limulus amebocyte lysate assay.

The authors added azide to prevent contamination by live bacteria under these pro-biotic conditions (a high glucose concentration at 37°C). They do not describe the use of sterilization filters before the 6 week incubation period nor after dialysis, which is a procedure with a risk of contamination. In our opinion, using sterilization filters is important in the process of AGE-preparation. But as mentioned earlier, the main problem is contamination by endotoxin.

In addition to the aforementioned concerns, the degree of glycation was only indirectly assessed as fluorescence measured by spectrophotometry. Although this indeed strongly suggests that AGEs have been formed, it would have been more elegant to show specific AGE levels such as \( N^\text{ε}(\text{carboxymethyl})\text{lysine} \) (CML) and \( N^\text{ε}(\text{carboxyethyl})\text{lysine} \) (CEL).

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**Fig. 1.** (A) \( N^\text{ε}(\text{carboxymethyl})\text{lysine} \) (CML) measured by western blot analysis, 1: BSA, non-incubated, 2: BSA incubated for 6 weeks at 37°C, 3: BSA incubated for 6 weeks at 37°C with 0.5 M glucose. (B) CML and (C) \( N^\text{ε}(\text{carboxyethyl})\text{lysine} \) (CEL) levels measured by liquid chromatography-tandem mass spectrometry.
The authors used the correct control preparation which was treated identically, with the exception that glucose was omitted. In our lab, we recently prepared AGEs by incubating endotoxin-free BSA 300 mg/ml at 37°C for 6 weeks with D-glucose (90 g/l) in a 0.4M phosphate buffer at pH 7.6. Interestingly, we saw an increase in CML and CEL in our control preparation, which was treated identically but without the addition of glucose, when compared with non-incubated BSA (Figure 1). It would have been interesting to use non-incubated endotoxin-free BSA as a control as well.

We conclude that Chang et al. propose a novel mechanism that could be involved in disturbances of sodium balance in diabetic nephropathy, i.e. an AGE-induced increase in expression of the epithelial sodium channel mRNA and protein, with enhanced sodium uptake in renal CCD cells. To substantiate this interesting hypothesis, it would be important to exclude endotoxin mediated effects.

Conflict of interest statement. None declared.

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Note: Dr Chang et al. have been invited to reply to this letter, but we did not receive an answer.

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Treatment with corticosteroids does not seem to benefit nephrogenic systemic fibrosis

Sir,

Nephrogenic systemic fibrosis (NSF) is a formerly unknown disease, which has become more and more recognized. It affects patients with kidney failure and, to a great extent, mimics systemic sclerosis [1]. An association between NSF and magnetic resonance image with gadolinium-based contrast agents has been suggested [2]. Herein, we report a case of NSF, in which corticosteroids were administered at the very beginning of the disease.

A 62-year-old male haemodialysis patient abruptly developed symmetrical painful, oedematous swelling of the skin on his fingers and palms. In addition, the swollen tissue around the palmar flexor tendons resembled tendovaginitis. The range of motion of the fingers was severely limited and painful. The patient received NSAIDs, but the symptoms persisted. Therefore, and because the disease was believed to be an inflammatory condition, the patient was treated with 50 mg prednisolone daily. However, swelling and pain did not improve. In the following weeks, the dosage of prednisolone was slowly tapered and finally discontinued after 2 months. As time passed, the oedematous swelling gradually resolved, but in parallel, the tissue became more and more fibrotic. After 1 year, the patient had contractions of his fingers, toes, elbows and knees (Figure 1). Given the course of the disease, we retrospectively recognized this condition to be NSF. Notably, 7 days before the start of symptoms, the patient had an MR angiography. The diagnosis was finally verified with a skin biopsy. The histological examination revealed large amounts of fibroblastic tissue, containing numerous CD34-positive fibroblasts [3].

NSF is a debilitating and sometimes fatal disease, affecting the skin, muscle, and internal organs [1,2]. These days, no consistently successful treatment exists. Because of their anti-oedematous and anti-fibrotic properties, corticosteroids may theoretically be helpful in the treatment of NSF. Given the ineffectiveness of prednisolone in our patient with regard to swelling, pain and progression of the disease, we believe that corticosteroids neither ameliorate the symptoms nor are they of benefit in the evolution of NSF.

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