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1 **An acute rise of plasma sodium concentration associates with syndecan-1 shedding**
2 **during hemodialysis**

3

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23 **Author contributions**

24 J. Koch, N.M.A. Idzerda, J. van den Born and C.F.M. Franssen conceived and designed the
25 study; E.M. Ettema, J. Kuipers and C.F.M. Franssen executed the clinical part of the study;
26 N.M.A. Idzerda and W. Dam performed the laboratory measurements; J. Koch, N.M.A.
27 Idzerda, J. van den Born and C.F.M. Franssen analyzed and interpreted the data and drafted
28 the manuscript. All authors approved the final version of the manuscript.

29

30 **Abstract**

31 Endothelial dysfunction (ED) contributes to the high incidence of cardiovascular events in
32 hemodialysis patients. Syndecan-1 in the endothelial glycocalyx can be shed into the
33 circulation serving as a biomarker for ED. As sodium is a trigger for glycocalyx shedding, we
34 now tested whether hemodialysis with higher dialysate sodium concentrations is associated
35 with more syndecan-1 shedding compared with standard hemodialysis (SHD).

36 In this cross-over study in 29 patients, plasma syndecan-1 was repeatedly measured during
37 SHD and during Hemocontrol hemodialysis (HHD) which is characterized by initially higher
38 dialysate and plasma sodium levels. Courses of syndecan-1 were compared with linear
39 mixed models. Syndecan-1 shedding was assessed by area under the curve analysis.

40 Plasma sodium increased early after the start of SHD and HHD, with higher values during
41 HHD (30 minutes: 142.3 mmol/L versus 139.9 mmol/L; $P < 0.001$). Syndecan-1 increased
42 significantly during both conditions but the percentage change was higher (42.9% versus
43 19.5%) and occurred earlier (120min versus 180min during) during HHD. Syndecan-1 levels
44 were significantly higher at 120 minutes during HHD compared to SHD ($P < 0.05$). Overall
45 syndecan-1 shedding was higher during HHD compared with SHD (means: 40.4 vs. 19.0
46 arbitrary units; $P = 0.06$). Lower predialysis plasma sodium and osmolality were associated
47 with greater intradialytic increases in syndecan-1 levels (both groups $P = 0.001$).

48 The rise in plasma syndecan-1 levels was more pronounced and occurred earlier during
49 hemodialysis with higher plasma sodium levels. Although we cannot prove that the rise in
50 plasma syndecan-1 originates from the endothelial glycocalyx, our findings are compatible
51 with sodium-driven endothelial glycocalyx-derived syndecan-1 shedding.

52

53 *Key words:* sodium; syndecan-1; hemodialysis.

54

55 **Introduction**

56 Hemodialysis (HD) patients have extremely increased cardiovascular (CV) morbidity and
57 mortality (39) and endothelial dysfunction (ED) is believed to have a major
58 pathophysiological role (16, 39, 45, 48). Several studies have shown that HD patients have
59 impaired endothelial function (12, 31) and markers of ED were found to predict survival of HD
60 patients (43). Vlahu et al. showed that dialysis patients had a loss of the endothelial
61 glycocalyx (eGC) thickness compared with healthy controls (52).

62 Previous studies suggest that shedding of syndecan-1 into the circulation may reflect
63 eGC degradation in HD patients (52). Syndecan-1, a transmembrane heparan sulfate
64 proteoglycan (HSPG) located in the endothelium, epithelium, hepatocytes and plasma cells,
65 is involved in regenerative growth, cellular adhesion and is an important constituent of the
66 pericellular coat or glycocalyx [reviewed in (47)]. Syndecan-1 shedding can be induced by
67 oxidative mechanisms (20, 21) and is stimulated under inflammatory conditions (2, 32, 54)
68 [reviewed in (3)]. More recent insights indicate that syndecan-1 may also play a role in
69 extrarenal non-osmotic sodium storage and that syndecan-1 shedding can occur due to
70 osmotic changes in plasma (23, 28) [reviewed in (30)] and alterations in volume status (19,
71 33). It has been suggested that the glycosaminoglycan domains of HSPGs in the
72 endothelium bind and osmotically inactivate circulating sodium ions, functioning as an
73 intravascular buffer compartment (23, 28) [reviewed in (30)]. At the same time and seemingly
74 in contrast to its function in sodium homeostasis, several studies have shown that the
75 endothelial glycocalyx is damaged in the presence of a sodium overload (35), probably
76 mediated by a reduction of heparan sulfate, impairing the endothelial buffer function and
77 thereby enhancing fluid retention (28, 38).

78 In patients on HD, ED is associated with co-morbidities such as diabetes and
79 hypertension [reviewed in (44)], but has also been shown to be induced by the HD treatment
80 itself (5, 26, 49). We recently found that, compared to healthy individuals, predialysis plasma
81 syndecan-1 levels were increased three-fold in patients on conventional HD and further
82 increased significantly during HD (22). This may be due to the HD-induced inflammatory

83 response and oxidative stress (42). Additionally, intradialytic changes in plasma sodium
84 concentration may induce ED and eGC shedding as previous studies suggest (28, 38).
85 Therefore, as constituent of the eGC (10), syndecan-1 could get shed into the circulation as
86 well. In most dialysis centers, patients are dialyzed with a fixed dialysate sodium
87 concentration of 139 or 140 mmol/L and, consequently, patients with a plasma sodium
88 concentration that is lower than the dialysate concentration will experience a rise in plasma
89 sodium concentration. In contrast to standard dialysis (SHD) with a fixed dialysate sodium
90 concentration, the Hemocontrol biofeedback system (HHD) is characterized by the use of
91 higher sodium levels in the first half of the HD session based on the concept that higher
92 sodium levels enhance the osmotic pressures and increase the capillary refill rate, thus
93 facilitating a higher ultrafiltration rate (UFR). Various studies have shown that this system
94 improves intradialytic hemodynamic stability (4, 17, 37) which is generally explained by its
95 effect on blood volume (4, 36).

96 The aim of our study was the relationship between plasma sodium and plasma
97 syndecan-1 which we studied during HHD and during SHD. We hypothesized that the
98 temporary rise in plasma sodium concentrations early during HHD leads to more ED. We
99 therefore asked the question whether ED, represented by glycocalyx shedding, was higher
100 during HHD compared to SHD, and measured plasma syndecan-1 as the principal outcome
101 parameter (thought to originate from the endothelial glycocalyx).

102

103 **Patients and Methods**

104 *Study protocol*

105 In this post-hoc analysis of the cross-over study from Ettema et al. (15). The current work is
106 covered under the previous IRB approval of this study (15). 29 patients on maintenance HD
107 were studied during a single SHD and a HHD dialysis session in randomized sequence.
108 Details of the study have been described before (15). In short, the study population consisted
109 of clinically stable HD patients who were on a thrice weekly HD schedule. Study-related HD
110 sessions took place in the morning or in the afternoon and lasted 4 hours. The
111 measurements took place at the first HD treatment of the week because the UF volume and
112 the blood volume decreases are most pronounced after the longest interdialytic interval (15).
113 The maximum time interval between the two treatments was 2 weeks. Treatment conditions
114 were identical during both treatments except for the dialysate sodium concentration which is
115 the major difference between SHD and HHD (vide infra). Medication use was similar at both
116 treatments. Patients were asked to refrain from drinking caffeine-containing beverages and
117 not to eat starting the night before the study sessions until 1 hour intra-dialysis. Patient
118 position during dialysis (half-supine) was standardized to exclude an effect of posture
119 changes on the circulation. Residual renal function was defined as diuresis of >200 mL per
120 24 hours.

121 Blood samples were collected from the arterial line at 6 moments during the dialysis
122 procedure: at the start of dialysis, after 30 minutes, after 60 minutes, after 120 minutes, after
123 180 minutes and after 240 minutes (end of dialysis). At these same time points, the change
124 in blood volume and cumulative ultrafiltration (UF) volume were registered. Blood pressure
125 and heart rate were measured every 30 minutes. Patients were treated with a single (patient-
126 dependent) dose of intravenous nadroparin, which was given immediately after start of the
127 dialysis procedure via the extracorporeal system before the dialyzer.

128

129

130 *HD treatment*

131 Both SHD and HHD were conducted on an Artis HD machine (Gambro Lundia AB, Lund,
132 Sweden) with a low-flux polysulphon dialyzer F8 or F10 (Fresenius Medical Care, Bad
133 Hamburg, Germany). The UF volume is the total volume of fluid that is removed during the
134 entire dialysis session and was set to achieve dry weight at the completion of the HD
135 session. UFR was expressed as L/h. Prescriptions regarding dry weight were made by the
136 nephrologists during their weekly visit to the participating patients. Dry weight was evaluated
137 clinically (peripheral edema, signs of pulmonary congestion, intradialytic and interdialytic
138 blood pressure course) in combination with the cardiothoracic ratio on chest radiography.

139 Blood flow and dialysate flow rates were 300 to 400 mL/min and 500 to 700 mL/min,
140 respectively and dialysate temperature was 36.0 or 36.5 °C. These settings were identical for
141 the individual patient at both treatments. Dialysate composition for SHD was sodium 139
142 mmol/L, magnesium 0.5 mmol/L, chloride 109 mmol/L, bicarbonate 34 mmol/L, acetate 3.0
143 mmol/L and glucose 1.0 g/dL. Dialysate potassium concentration varied between 1 and 3
144 mmol/L and calcium varied between 1.25 and 1.50 mmol/L depending on the prevailing
145 plasma potassium and calcium concentration. Treatment conditions were identical for the
146 individual patient during both treatments, except for the dialysate sodium concentration.
147 During SHD, the dialysate conductivity was 13.9 mS/cm throughout the dialysis session.
148 During HHD, the equivalent conductivity was set at 13.9 mS/cm, indicating an identical net
149 sodium removal compared with SHD, with lower- and upper tolerance limits for dialysate
150 sodium of 13.3 and 16.0 mS/cm, respectively. With HHD, large and sudden decreases in
151 blood volume are prevented in order to improve intradialytic hemodynamic stability. To this
152 end the patients' blood volume is guided along a predefined ideal relative blood volume
153 trajectory by continuously adjusting UF volume and dialysate conductivity. The pre-set ideal
154 blood volume curve has a marked decrease in the beginning of the dialysis session, whereas
155 it is more stable during the second half of the treatment (37). Hallmark of HHD is the
156 combination of a higher UFR and higher dialysate conductivity during the first half of the
157 dialysis session. This results in higher plasma sodium levels during the first half of the
158 dialysis session and a more pronounced initial decrease in blood volume. Since HHD uses

159 higher UFRs during the first half of treatment, lower UFRs can be used during the second
160 half of the dialysis session, which hemodynamically is considered to be the most critical part
161 of the treatment.

162

163 *Laboratory procedures*

164 Blood samples for sodium, potassium, urea and osmolality were collected in heparin-coated
165 tubes. Plasma sodium and potassium were measured with the indirect method of ion-
166 selective electrode on a Roche Modular (Hitachi, Tokyo, Japan). Urea was measured with
167 the colorimetric method on a Roche Modular analyzer. Plasma osmolality was measured by
168 freezing-point depression on the Osmostat Osmometer (Arkray, Kyoto, Japan). Blood
169 samples for the determination of syndecan-1 were collected in EDTA tubes and immediately
170 put on ice. Next, the samples were centrifuged and stored at -20°C , thawed once and then
171 stored at -80°C until procession. Syndecan-1 concentrations were measured in EDTA
172 plasma samples, using sCD138 sandwich ELISA kits (Diaclone, Besancon, France)
173 according to manufacturer's instructions with standard line on each plate.

174

175 *Correction for hemoconcentration*

176 Considering the Sieving characteristics of low-flux polysulphone artificial dialyzer and
177 according to the criteria proposed by the Uremic Toxin Work Group, molecules with a
178 molecular weight between 500 and 6000 Da are presumably only partially or not at all
179 removed with HD (34, 53). Since syndecan-1 has a molecular weight of ≈ 77.000 Da it is
180 unlikely that it is removed by dialysis. Indeed, we did not detect measurable syndecan-1 in
181 the dialysate of the first 10 patients in this study (the lower detection range of our analysis
182 was 8 ng/mL). Therefore, we concluded that syndecan-1 is not removed by HD and,
183 consequently plasma levels of syndecan-1 were corrected for hemoconcentration according
184 to reference (40).

185

186 *Statistical analysis*

187 Analyses were performed with SPSS version 20.0, GraphPad Prism version 7.00 (GraphPad
188 Software, La Jolla, CA, USA) and R version 3.3.1 (R Foundation for Statistical computing).
189 Results are expressed as mean \pm SD, median [IQR] or mean (95% CI) when appropriate.
190 Normality was tested with the Shapiro-Wilk test. A (non-parametric) Levene's test was used
191 to verify the equality of variances in the data ($P>0.05$). Comparisons were made with a
192 Wilcoxon Signed Rank Test, a paired T-test or Fisher's exact test when appropriate.
193 Relationships of sodium and osmolality with syndecan-1 levels over time were assessed with
194 a repeated measures mixed effects model. Here, the primary outcome measure was plasma
195 syndecan-1. The models were adjusted for age, sex, systolic and diastolic blood pressure,
196 body weight and duration of dialysis. Patient number was added as random effect.
197 Cumulative intradialytic shedding of syndecan-1 was assessed by area under the curve
198 analysis. Correlations between interval variables were calculated using the Pearson's
199 correlation coefficient. Relationships of sodium with syndecan-1 shedding, as well as
200 syndecan-1 shedding and total UF volume were assessed by univariate analysis.

201

202 **Results**

203 *Patients*

204 The patient characteristics are summarized in **Table 1**. The study population included 21
205 men and 8 women, the mean age was 63.4 (\pm 17.0) years and the dialysis vintage was 33.9
206 (\pm 27.0) months. As much as 52% of patients had a history of a previous CV event and 52%
207 had residual renal function. There was a small but significant difference in predialysis plasma
208 syndecan-1 levels between SHD (59.5 ng/mL; IQR 33.5 to 88.0) and HHD (51.0 ng/mL; IQR
209 26.0 to 87.0). Other baseline laboratory parameters, predialysis and postdialysis weight and
210 blood pressure were comparable for the two treatments (**Table 2**).

211

212 *Course of plasma sodium concentration*

213 As expected, plasma sodium levels were significantly higher during the first half of HHD
214 compared with SHD and the difference was already present at 30 minutes into the dialysis
215 procedure (139.9 mmol/L [95%CI 138.9 to 140.8] during SHD versus 142.3 mmol/L [95%CI
216 141.2 to 143.3] during HHD; $P < 0.001$) and remained significant until at least 120 minutes
217 after the start of HD (139.3 mmol/L [95% CI 138.7 to 139.9] versus 140.1 mmol/L [95% CI
218 139.4 to 140.9; $P < 0.05$]). At the end of the HD session, plasma sodium was significantly
219 lower with HHD compared with SHD (139.9 mmol/L [95%CI 139.2 to 140.6] during SHD
220 versus 138.5 mmol/L [95%CI 137.7 to 139.2] during HHD; $P < 0.01$) (**Table 3**). The UFR in
221 HHD was significantly higher at 60 minutes (0.75 vs. 0.62 L/h; $P < 0.001$) and lower at 240
222 minutes (0.54 vs. 0.67 L/h; $P < 0.001$) compared to SHD (data not shown).

223

224 *Course of plasma syndecan-1 levels*

225 Plasma syndecan-1 levels increased significantly during both SHD and HHD (**Table 3 and**
226 **Figure 1**). However, the rise in syndecan-1 levels during HHD was more pronounced (peak
227 concentration compared with baseline: +42.9% [95%CI 21.1 to 64.6] versus +19.5% [95%CI
228 17.3 to 31.6]; $P = 0.08$) and occurred earlier (120 minutes versus 180 minutes). The increase
229 in plasma syndecan-1 levels was significantly higher at 120 minutes during HHD compared

230 to SHD ($P < 0.05$) (**Table 3 and Figure 1**). Cumulative shed syndecan-1 as estimated with
231 area under the curve analysis was higher during HHD (40.4 [95%CI 15.8 to 65.1]) compared
232 with SHD (19.0 [95%CI 2.9 to 35.1]), albeit at borderline significance ($P = 0.06$).

233

234 *Lower predialysis sodium and osmolality predict the intradialytic change in syndecan-1 levels*

235 Plasma sodium level and osmolality before dialysis were independent predictors of
236 syndecan-1 levels during dialysis. Lower sodium levels at baseline were associated with a
237 larger intradialytic increase of syndecan-1: one unit (mmol/L) lower predialytic sodium level
238 was associated with an additional increase in syndecan-1 during dialysis of 4.4% [95%CI 0.9
239 to 7.9; $P = 0.02$] at 30 minutes into the dialysis session. Similarly, lower predialytic osmolality
240 was associated with a more pronounced increase in syndecan-1 during dialysis: one unit
241 (mosmol/kg) lower osmolality was associated with an additional increase in syndecan-1
242 during dialysis of 2.6% [95%CI 0.8 to 4.4; $P < 0.01$] at 30 minutes of HD. Additional analyses,
243 adding potassium or urea to the mixed effect model did not change these results.

244

245 *Cumulative ultrafiltration volume predicts total intradialytic syndecan-1 shedding in HHD but*
246 *not in SHD*

247 In univariate analysis, a higher total UF volume tended to be associated with a higher degree
248 of intradialytic syndecan-1 shedding ($R^2 = 0.085$; $P = 0.04$). When SHD and HHD were
249 analyzed separately, a similar correlation was observed in HHD ($R^2 = 0.135$; $P = 0.05$), but not
250 in SHD ($R^2 = 0.005$; $P = 0.70$) (**Figure 2**).

251 Multivariate analysis showed that total UF volume independently predicted total intradialytic
252 syndecan-1 shedding during dialysis. Patients with higher total UF volume during dialysis
253 had higher AUC values for syndecan-1 (AUC increase of 3.5 [95%CI 0.8 to 6.2]; $P = 0.03$, per
254 0.1 L increase in total UF volume). Total UF volume predicted syndecan-1 AUC in HHD
255 (AUC increase of 6.2 [95%CI 2.1 to 10.3]; $P = 0.01$) but not in SHD (AUC increase of 1.5
256 [95%CI -2.4 to 5.4]; $P = 0.48$). In the mixed effects model, the association between total

257 ultrafiltration volume and syndecan-1 was not influenced by the presence of residual renal
258 function (P=0.255).
259

260 **Discussion**

261 The main finding of this study was that the rise in plasma syndecan-1 levels was more
262 pronounced and occurred earlier during HD with higher plasma sodium levels. Cumulative
263 syndecan-1 shedding tended to be higher during HHD. Our study is the first to show this
264 effect of higher plasma sodium levels on plasma syndecan-1 in HD patients. Although we
265 cannot prove that the rise in plasma syndecan-1 originates from the endothelial glycocalyx,
266 our findings are compatible with sodium-driven endothelial glycocalyx-derived syndecan-1
267 shedding.

268 Plasma syndecan-1 levels not only increased during HHD but also during SHD
269 confirming our previous findings in SHD patients (22). Acute rises in plasma syndecan-1
270 levels can be elicited by inflammatory and oxidative stimuli. Both stimuli are inherent to the
271 HD procedure due to the bioincompatibility reaction that results from the contact between
272 blood and the extracorporeal system. The present study shows that the intradialytic course of
273 plasma sodium levels is an additional and modifiable factor.

274 Syndecan-1 shedding after an acute inflammatory insult (such as the HD procedure)
275 is considered to originate from the endothelium (47) among other cells/tissues and to reflect
276 glycocalyx damage by being cleaved and shed into the circulation (2, 7, 13, 52). Although
277 syndecan-1 is present in the glycocalyx of multiple different cell types, its location and
278 shedding from the endothelial glycocalyx is a possibility that has been shown by previous
279 researchers (38). We conclude that higher plasma sodium concentrations in HD patients are
280 associated with an earlier and more pronounced intradialytic rise in plasma syndecan-1
281 levels. Referring to the literature, it is likely that (among other tissues) this syndecan-1
282 shedding takes place from the endothelial glycocalyx. This is in accordance with *in vitro* data
283 using endothelial cells in culture. These in-vitro studies have shown that the stability of the
284 eGC can be influenced by sodium. Here, incubation of human endothelial cells (EA.hy926)
285 with human plasma containing high sodium levels (147 mM or + 2–5 mM) led to the collapse
286 of the eGC resulting in endothelial stiffening as measured with atomic force microscopy (28).
287 In fact, next to a reduction in the glycocalyx component heparan sulfate in

288 immunofluorescence stainings, syndecan-1 was also found to be increased in the
289 supernatant upon sodium overload (38). This indicates that sodium is a factor in glycocalyx
290 breakdown and cleavage of syndecan-1. Moreover, research has shown that high sodium
291 levels cause a down-regulation of nitric oxide formation in endothelial cells, again indicating
292 ED (6, 8, 29). Also human studies in normotensive men have shown that intravenous sodium
293 loading has a direct damaging effect of the endothelial surface layer (35).

294 In our study, lower predialytic plasma sodium concentrations and osmolality were
295 associated with greater intradialytic increases in syndecan-1 levels. This could indicate that
296 lower predialytic plasma sodium levels provide a stronger stimulus for syndecan-1 shedding
297 and thus ED. Alternatively, lower predialytic plasma sodium concentrations could reflect
298 diluted plasma sodium levels due to higher intravascular fluid volume as a result of
299 overhydration. Volume overload could be responsible for syndecan-1 shedding which is in
300 line with previous research findings (9). Dekkers et al. reported an association between
301 moderate and severe fluid overload and lower survival in HD patients which was linked to
302 inflammation (11). Further studies explored that volume overload can lead to ED next to
303 inflammation, which has also been reported in HD-dependent patients with CKD (27). This is
304 in accordance with our finding that higher total UF volume tended to be associated with a
305 higher degree of intradialytic syndecan-1 shedding in the HHD modality. Again, we think this
306 association is only found in the HHD group because of the higher initial sodium load that
307 potentiates syndecan-1 shedding.

308 Several studies have shown the detrimental effects of excessive sodium intake which
309 has been linked to the expansion of extracellular volume and hypertension. Various studies
310 showed evidence that low sodium intake reduces blood pressure in both normotensive
311 individuals and hypertensive patients. (1, 18) Interestingly, subjects with a long term stable
312 sodium diet showed changes in total body sodium that were not related to changes in
313 extracellular volume or body weight (52). This advocates the presence of a buffer where
314 sodium can be stored in a non-osmotic way. Studies on marine invertebrates versus
315 vertebrates have shown that the more a subject is exposed to sodium, the more sulfated

316 GAGs would be required (14, 50). In humans, such highly sulfated heparan sulfated GAGs
317 are especially found in tissues that serve as a barrier, such as the skin, the lungs and the
318 endothelium. In the skin, the inactivation of sodium by binding to GAGs in a non-osmotic
319 way has already been shown by Titze et al. (46) The highly sulfated negatively charged
320 GAGs in the endothelial glycocalyx may have similar sodium-binding properties. In contrast
321 to the skin interstitium, the endothelial glycocalyx is in direct contact with circulating sodium
322 and could therefore serve as a buffer before it enters the interstitium. In vitro experiments
323 using sodium nuclear magnetic resonance have shown that sodium binds reversibly to GAGs
324 in the endothelial glycocalyx under flow (41). The negatively charged GAGs may attract
325 positively charged sodium ions which form the most abundant group of cations in the blood
326 (41). Vlahu et al. have shown that elevated levels of plasma syndecan-1, reflecting
327 glycocalyx breakdown, has been associated with increased need for ultrafiltration (51).
328 According to these data, GAGs in the endothelial glycocalyx seem to play a role in sodium
329 homeostasis. Next to that, there seems to be a detrimental effect of sodium on the
330 endothelial glycocalyx as well, which has been shown by Oberleithner et al. (28) and other
331 research groups (35, 38). So far, no study has investigated yet how this seemingly paradox
332 is related. We speculate that one underlying mechanism could simply be an overload of
333 sodium in the endothelial glycocalyx which possibly results in a breakdown or collapse.

334 There are important limitations in our study. Although this is the largest study
335 comparing courses of syndecan-1 during different salt conditions, the study population is still
336 relatively small. Future studies with greater power have to be performed before definite
337 conclusions can be drawn. As this is a short-term study of the effects of a temporary
338 intradialytic increase in plasma sodium, we cannot extrapolate the findings to long-term
339 treatment with HHD. Therefore, the clinical implications of the more pronounced increase in
340 syndecan-1 levels during HHD are still unknown. This should be addressed in future studies
341 using robust clinical outcome parameters, for instance data on arterial stiffness (24, 25) and
342 functional ED tests, CV events and survival, as well as markers for inflammation and the
343 endothelium.

344 In conclusion, the rise in plasma syndecan-1 levels was more pronounced and occurred
345 earlier during hemodialysis with higher plasma sodium levels. Although we cannot prove that
346 the rise in plasma syndecan-1 originates from the endothelial glycocalyx, our findings are
347 compatible with sodium-driven endothelial glycocalyx-derived syndecan-1 shedding.

348 Our findings also suggest that the HD procedure itself may contribute to ED in dialysis
349 patients. Further research should explore the pathophysiology and clinical implications in
350 further depth and in a larger cohort.

351

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354 Graduate School of Medical Sciences of the University Medical Center Groningen.

355

356 **Disclosures**

357 None to declare.

358

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531 **Figure 1. Levels of plasma sodium and plasma syndecan-1.**

532 Courses of plasma sodium (dotted line) and plasma syndecan-1 (solid line) during SHD (A;
533 left panel) and during HHD (B; right panel). Values are presented as mean for sodium and
534 percentage change for plasma syndecan-1; error bars indicate 95% confidence intervals.

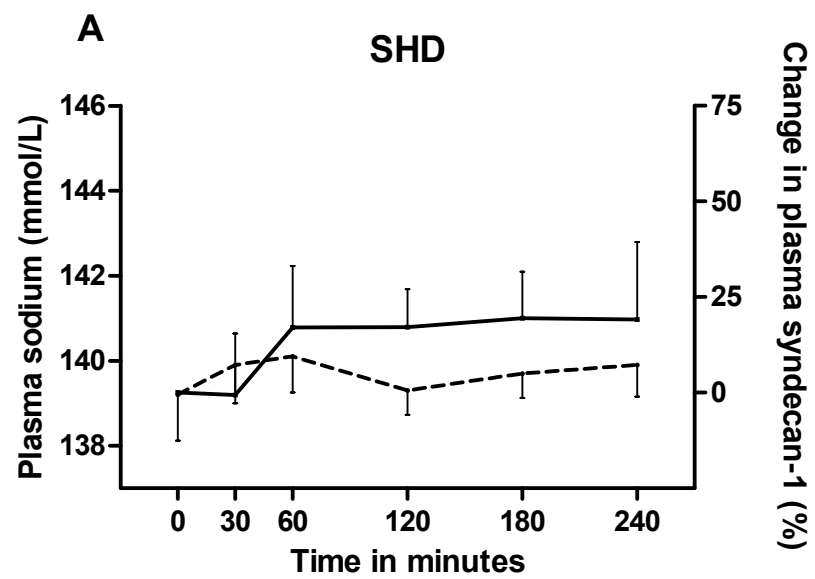
535 * : P-value of <0.05, ** : P-value <0.01, *** : P-value <0.001 for the difference in plasma
536 sodium between SHD and HHD. ○ : Indicates P-value of <0.05 for the difference in plasma
537 syndecan-1 level between SHD and HHD.

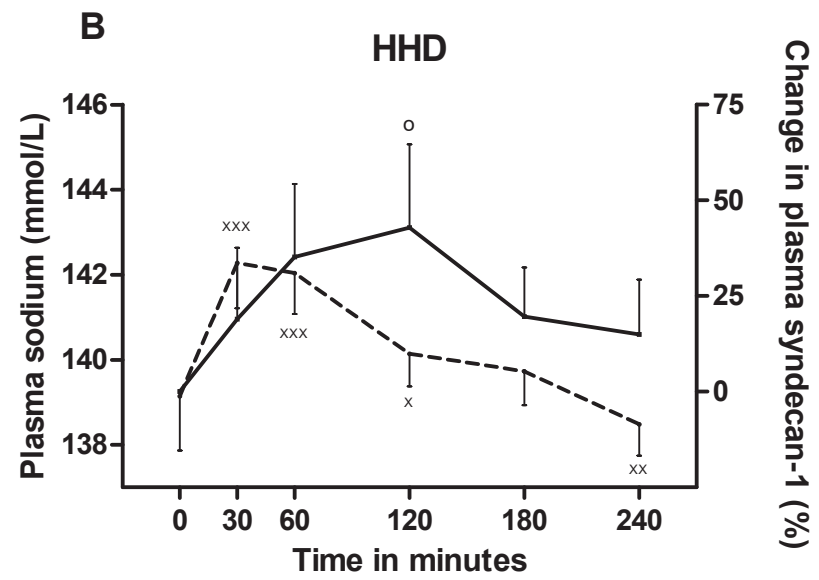
538

539 **Figure 2. Relationship between the total UF volume and total syndecan-1 shedding.**

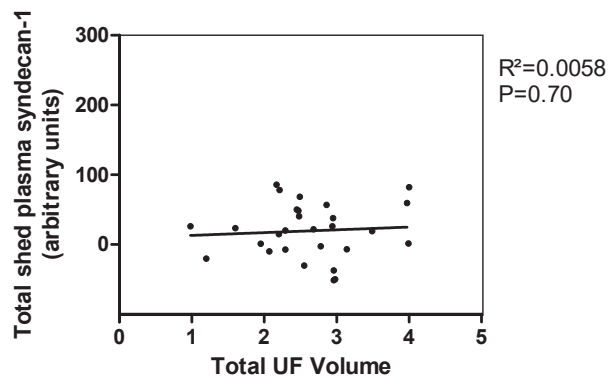
540 Univariate relationship between total shed plasma syndecan-1 and total UF volume during

541 SHD (A; left panel) and HHD (B; right panel).



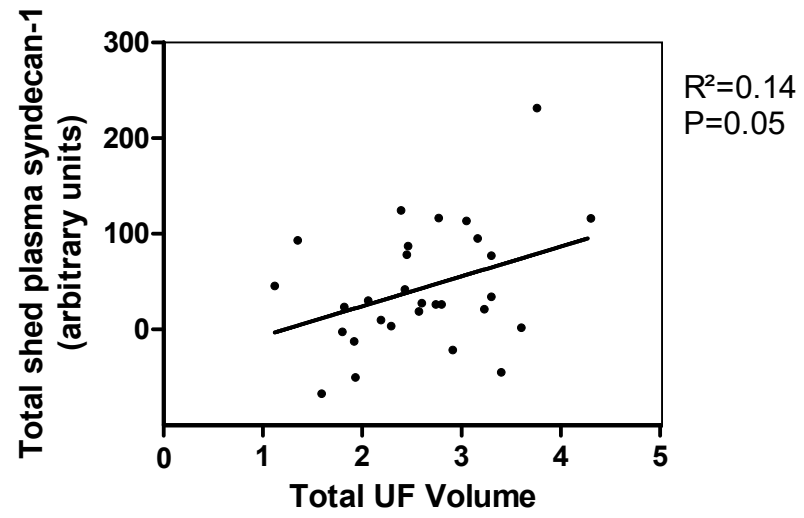


A **SHD**



B

HHD



1 **Table 1. Patient characteristics.**

Number of patients	29
Females, n (%)	8 (28)
Age, y	63.4 ± 17.0
Dialysis vintage, m	33.9 ± 27.0
Residual renal function (RRF),	
Proportion of patients with RRF, n (%)	15 (51.7)
RRF, ml/min	1 (0.0 to 3.1)
Cardiovascular history, n (%)	15 (52.0)
Diabetes mellitus, n (%)	8 (28.0)
Hypertension, n (%)	26 (90.0)
Cause of renal failure, n (%)	
Hypertension	8 (28.0)
Glomerulonephritis	4 (14.0)
Diabetes mellitus	3 (10.0)
Hydronephrosis	2 (7.0)
Other	6 (21.0)
Unknown	6 (21.0)
Concomitant medication, n (%)	
Statins	9 (31.0)
Antihypertensive drugs	23 (79.0)
Immunosuppressive drugs	4 (14.0)
Insulin	7 (24.0)

2

3 Data are shown as means ± SD, except for RRF which is shown as medians with
 4 interquartile ranges in parentheses. Categorical distributed variables are shown as numbers
 5 and percentages [*n* (%)]. Abbreviations: m, months; n, number; RRF, residual renal function;
 6 y, years.

1 **Table 2. Characteristics for SHD and HHD.**

	SHD	HHD	P-value*
Sodium, mmol/L Predialysis	139.0 ± 3.0	139.0 ± 3.4	0.90
Plasma syndecan-1, ng/mL Predialysis	59.0 (33 to 88)	51.0 (26.0 to 87.0)	0.023
Plasma potassium, mEq/L Predialysis	4,8 (4,6 to 5,3)	4,8 (4,5 to 5,1)	0,803
Plasma urea, mmol/L Predialysis	27,1 (20,8 to 31)	26,8 (19,7 to 30,6)	0,973
Osmolality, mosm/kg Predialysis	285.0 ± 5.7	286.0 ± 6.1	0.85
Ultrafiltration volume, L	2.6 ± 0.7	2.6 ± 0.7	0.95
Weight, kg			
Predialysis	82.1 ± 16.0	82.3 ± 16.0	0.96
Postdialysis	80.0 ± 16.0	80.1 ± 16.0	0.50
Systolic BP, mmHg			
Predialysis	136 (121 to 147)	137.0 (122 to 150)	0.62
Postdialysis	135 (114 to 147)	139 (120 to 153)	0.54
Diastolic BP, mmHg			
Predialysis	73 ± 12	74 ± 11	0.87
Postdialysis	72 ± 12	71 ± 12	0.83

2

3 Data are shown as means ± SD, except for plasma syndecan-1 and systolic BP which are
 4 shown as medians with interquartile ranges in parentheses. Abbreviations: BP, blood
 5 pressure; HHD, Hemocontrol hemodialysis; kg, kilogram; L, liter; mmHg, millimeter of
 6 mercury; mmol/L, millimol per liter; mosm/kg, milliosmol per kilogram; ng/mL, nanogram per
 7 milliliter; SHD, standard hemodialysis. * P-value for difference at baseline between SHD and
 8 HHD.

1 **Table 3. Change in plasma sodium and percentage change in plasma syndecan-1**
 2 **levels during SHD and HHD.**

	SHD		HHD		SHD versus HHD
Time	Plasma sodium, mmol/L				
		P-value*		P-value*	P-value
0 min	139.2 [138.1, 140.4]		139.1 [137.9, 140.4]		
30 min	139.9 [138.9, 140.8]	0.0299	142.3 [141.2, 143.3]	<0.0001	<0.0001
60 min	140.1 [139.3, 140.9]	0.0130	142.0 [141.1, 142.9]	<0.0001	<0.0001
120 min	139.3 [138.7, 139.9]	0.7259	140.1 [139.4, 140.9]	0.0523	0.037
180 min	139.7 [139.1, 140.1]	0.2673	139.7 [138.9, 140.5]	0.3296	0.971
240 min	139.9 [139.2, 140.6]	0.4441	138.5 [137.7, 139.2]	0.3956	0.002
Time	Plasma syndecan-1, percentage change				
		P-value*		P-value*	P-value
0 min	0		0		
30 min	-0.6 [-16.8, 15.5]	0.678	19.0 [0.3, 37.6]	0.007	0.313
60 min	17.0 [0.9, 33.1]	0.162	35.2 [16.2, 54.3]	<0.001	0.069
120 min	17.2 [7.3, 27.1]	0.003	42.9 [21.1, 64.6]	<0.001	0.029
180 min	19.5 [7.3, 31.6]	0.016	19.7 [6.8, 32.5]	0.026	0.891
240 min	19.1 [-1.1, 39.4]	0.515	14.9 [0.6, 29.3]	0.223	0.701

3

4 Results for sodium are presented as mean [95% confidence interval]. Results for plasma
 5 syndecan-1 are presented as percentage change from 0 min [95% confidence interval]. * P-
 6 value for percentage difference in syndecan-1 levels and absolute difference in sodium levels
 7 compared to baseline (0 min) within dialysis modalities. Abbreviations: HHD, Hemocontrol
 8 hemodialysis; min, minutes; mmol/L, millimol per liter; SHD, standard hemodialysis.