

Endurance Exercise Intervention Is Beneficial to Kidney Function in a Rat Model of Isolated Abdominal Venous Congestion: a Pilot Study

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1 **Endurance exercise intervention is beneficial to kidney function in a rat model of isolated**  
2 **abdominal venous congestion: a pilot study**

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47 **Abbreviations**

48	ALT	Alanine aminotransferase
49	AST	Aspartate aminotransferase
50	ATIIT1R	Angiotensin II type 1 receptor
51	ATP	Adenosine triphosphate
52	AWT	Anterior wall thickness
53	BSA	Bovine serum albumin
54	CO	Cardiac output
55	CRP	C-reactive protein
56	CVP	Central venous pressure
57	EDV	End-diastolic volume
58	EF	Ejection fraction
59	ESV	End-systolic volume
60	FE <sub>Na</sub>	Fractional excretion of sodium
61	G	Gauge (needle diameter)
62	GFR	Glomerular filtration rate
63	HPD	High protein diet
64	HR	Heart rate
65	IVCc	Inferior vena cava constriction
66	IVCc-MOD	IVCc rats subjected to moderate intense endurance exercise
67	IVCc-SED	Sedentary IVCc rats
68	KIM-1	Kidney injury molecule 1
69	LVEDD	Left ventricular end-diastolic diameter
70	LVEDP	Left ventricular end-diastolic pressure
71	LVESD	Left ventricular end-systolic diameter
72	LVP	Left ventricular pressure
73	NO	Nitric oxide
74	PWT	Posterior wall thickness
75	RFR	Renal functional reserve
76	TBS-T	Tris-buffered solution containing 0.1% Tween-20

77 **Abstract**

78 In this study, the effects of moderate intense endurance exercise on heart and kidney function and  
79 morphology were studied in a thoracic inferior vena cava constricted (IVCc) rat model of abdominal  
80 venous congestion.

81 After IVC surgical constriction, eight sedentary male Sprague-Dawley IVCc rats (IVCc-SED) were  
82 compared to eight IVCc rats subjected to moderate intense endurance exercise (IVCc-MOD). Heart and  
83 kidney function were examined and renal functional reserve (RFR) was investigated by administering  
84 a high protein diet (HPD). After twelve weeks of exercise training, abdominal venous pressure, indices  
85 of body fat content, plasma cystatin C levels and post-HPD urinary KIM-1 levels were all significantly  
86 lower in IVCc-MOD versus IVCc-SED rats ( $P<0.05$ ). RFR did not differ between both groups.

87 The implementation of moderate intense endurance exercise in the IVCc model reduces abdominal  
88 venous pressure and is beneficial to kidney function.

89 **Statement clinical relevance**

90 This study provides relevant insight concerning the impact of moderate intense endurance exercise  
91 training on the development of abdominal venous congestion and the progression of kidney  
92 dysfunction.

93 **Introduction**

94 Venous congestion is an established mechanism driving disease progression in heart failure (Mullens  
95 *et al.*, 2009; Dupont *et al.*, 2011; Nijst & Mullens, 2014). Nonetheless, the impact of isolated venous  
96 congestion on organ function, separate from cardiac dysfunction, remains to be elucidated in patients  
97 (Adams *et al.*, 2005; Damman *et al.*, 2010). Recently, Cops *et al.* developed a new rat model of isolated  
98 abdominal venous congestion (IVCc rat model) (Cops *et al.*, 2018b) and this is currently the most  
99 clinically relevant animal model for the study of congestion (Cops *et al.*, 2019a). In this IVCc model, it  
100 was demonstrated that isolated abdominal venous congestion is associated with retrogradely  
101 conducted glomerular hypertension, without major impact on the glomerular filtration rate (GFR), and  
102 with hepatic fibrosis. Both features are likely due to an upregulated inflammatory status (Cops *et al.*,  
103 2018a). Notably, cardiac function was not compromised. In this way, a clinically relevant and unique  
104 rat model was developed to study the effects of backward failure separately from forward failure.

105 To date, the management of heart failure patients demonstrating venous congestion is challenging  
106 and no unambiguous decongestive treatment is available. Consequently, congestive heart failure  
107 patients currently receive therapeutics proven to be suitable for either heart or kidney failure  
108 (Verbrugge *et al.*, 2014; Martens & Mullens, 2018). Moreover, unfavorable outcomes in many patients  
109 with heart failure are observed, thereby stressing the need for better therapy. In this way, the urgent  
110 need for specific treatment options to tackle venous congestion is highlighted.

111 Exercise training has emerged as an important intervention for heart and kidney failure. Exercise  
112 intervention is recommended by European and American guidelines in both hospitalized and stable  
113 chronic heart failure patients (O'Connor *et al.*, 2009; Lewinter *et al.*, 2015; Forestieri *et al.*, 2016;  
114 Groehs *et al.*, 2016). In accordance with heart failure, the benefits of exercise training have been  
115 demonstrated in chronic kidney disease (Howden *et al.*, 2012) and end-stage renal disease (Johansen,  
116 2007). However, little is known about the effects of exercise intervention in heart failure patients with  
117 congestion (Cops *et al.*, 2019b), demonstrating worsening renal function, since most clinical studies  
118 exclude heart failure patients with severe kidney injury.

119 The objective of this study was to implement moderate intense endurance exercise intervention in this  
120 rat model of selective abdominal venous congestion and to identify the effects of a training  
121 intervention on cardiac and renal morphology and function. We hypothesize that moderate intense  
122 endurance exercise training leads to a reduction in congestion and delays the progression of the kidney  
123 dysfunction observed in the IVCc rat model. This study offers the unique possibility of studying the  
124 effects of endurance exercise intervention on the pathophysiology of congestion.

## 125 **Methods**

### 126 *Ethical approval and housing*

127 The authors declare that all supporting data are available within the article. All applicable international,  
128 national, and/or institutional guidelines for the care and use of animals were followed. All procedures  
129 performed in this study involving animals were in accordance with the ethical standards of the  
130 institution at which the studies were conducted. This study conforms to the EU Directive 2010/63/EU  
131 for animal experiments and was approved by the Ethical Committee for Animal Experiments of Hasselt  
132 University, Belgium (protocol number: 201757). Animals were maintained in a temperature (22°C) and  
133 light (12:12h cycle) controlled animal facility and had *ad libitum* access to a normal pellet diet (18% fat,  
134 24% protein and 58% carbohydrate as a percentage of total kcal, 2018 Teklad global rodent diet,  
135 Harlan, Belgium) and water (Cops *et al.*, 2018a; Cops *et al.*, 2018b).

### 136 *Study design*

137 Forty male Sprague-Dawley rats (6±1 weeks, 135±15g, Charles River, France) were subjected to inferior  
138 IVC constriction to induce abdominal venous congestion (Cops *et al.*, 2018a; Cops *et al.*, 2018b). Only  
139 male rats were used to exclude the influence of the female reproductive system. Five rats died due to  
140 respiratory arrest during intubation and nineteen rats died due to consequences of the constriction  
141 immediately after application of the constriction, resulting in a peri-operative mortality rate of 54%  
142 (19/35). The sixteen surviving rats were randomly divided into two groups: IVCC sedentary rats (IVCC-  
143 SED group, n=8) were compared to IVCC rats subjected to moderate intense endurance exercise (IVCC-  
144 MOD group, n=8) and were studied for a period of 12 weeks to allow comparison with our previous  
145 study (Cops *et al.*, 2018b). In week 13, the renal functional reserve (RFR) was investigated using a high  
146 protein diet (HPD). Blinding was not possible due to practical reasons.

147 Sham groups (sham-SED and sham-MOD) were not included because the aim of this study was to  
148 investigate if exercise training reduces abdominal venous congestion, thereby delaying kidney  
149 dysfunction. Moreover, sham-SED rats were described in previous papers of Cops *et al.* (2018) (Cops  
150 *et al.*, 2018a; Cops *et al.*, 2018b) and the effects of moderate intense exercise training on general  
151 health and kidney function in healthy rats, the so-called sham-MOD group, has already been described  
152 extensively in literature (Zheng *et al.*, 2006; Cao *et al.*, 2016; Li & Wang, 2017).

### 153 *Experimental protocol*

154 Surgical constriction of the IVC was applied as described before (Cops *et al.*, 2018a; Cops *et al.*, 2018b).  
155 Briefly, under isoflurane anesthesia (1.5% volume supplemented with oxygen) and after intubation or  
156 tracheotomy, a right anterolateral thoracotomy was performed and the IVC was dissected from the

157 surrounding tissue. A permanent constriction was applied by tying a surgical wire (6-0 prolene, VMD,  
158 Belgium) around the IVC and a 20G needle, after which the 20G needle was removed and the wound  
159 was closed. Meloxicam (1 mg/kg, Boehringer, Germany) was administered subcutaneously pre-  
160 operatively and was continued post-operatively twice a day for three consecutive days. Antibiotics (10  
161 mg/kg/day, Baytril, Bayer, Belgium) were administered via the drinking water to both groups for five  
162 consecutive days postoperatively. In the first week after surgery, IVCC-MOD rats were enrolled in a  
163 treadmill running program. After twelve weeks of exercise training, rats were weighed, blood samples  
164 were obtained immediately after exercise training from the tail artery under isoflurane anesthesia (1.5-  
165 2% volume supplemented with oxygen), 24h urine samples were collected using standard rodent  
166 metabolic cages (technilab-BMI, the Netherlands) and echocardiography was performed (Cops *et al.*,  
167 2018a; Cops *et al.*, 2018b).

168 In week 13, rats were fed a HPD for four consecutive days to investigate RFR. Again, blood samples  
169 were obtained from the tail artery under isoflurane anesthesia and 24h urine samples were collected  
170 using metabolic cages. Next, invasive hemodynamic measurements were performed. Afterwards, rats  
171 were sacrificed with an overdose of pentobarbital (200 mg/kg, i.p.) and the degree of constriction was  
172 visually confirmed in each rat. Kidneys, liver and heart were excised for weighing and further  
173 histological and molecular examination. Tissues were fixed overnight in 4% paraformaldehyde and  
174 transferred to 70% ethanol until embedding in paraffin. Residual tissues were crushed into fine  
175 powder, snap frozen in liquid nitrogen and stored at -80°C (Cops *et al.*, 2018a; Cops *et al.*, 2018b).

#### 176 *Moderate intense endurance exercise intervention*

177 Moderate intense endurance exercise in rats was defined as daily treadmill running at 11m/min and  
178 at a 15° inclination for 1h starting at 8 a.m., as described previously by Wens *et al.* (2015) (Wens *et al.*  
179 *et al.*, 2015). During habituation animals, were familiarized with treadmill running (week 1 to 3) at  
180 progressively increased training durations, intensities and inclination. At the start of week 3, rats were  
181 able to run at the preconceived goals. Animals were encouraged to run by means of a gentle tap and  
182 not by electrical shocks since this causes stress. Sedentary rats were subjected to similar daily handling,  
183 except for the exercise protocol. Training efficacy was assessed by 24h food and water intake obtained  
184 by the use of metabolic cages and by plasma lactate levels. Plasma lactate levels at baseline and after  
185 12 weeks of follow-up were determined from capillary tail blood collection, immediately after  
186 cessation of the exercise training (GMRD-054, Analis SA, Belgium) using an Analox GM7 (Analis SA,  
187 Belgium). Baseline plasma levels of IVCC-SED rats were not available due to technical errors.

#### 188 *Blood and urine biochemical analysis*

189 Blood samples were centrifuged (2000 rpm, 10 min) and plasma was preserved (-20°C) for later  
190 analysis. Plasma samples were analyzed for triglycerides, creatinine, cystatin C, urea, alanine  
191 aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, C-reactive protein (CRP) and  
192 aldosterone using an automated analyzer (Cobas 8000 ISE module and Cobas 8000 c702 and c502  
193 module, Roche diagnostics, Germany) (Erdem *et al.*, 2000; Vaidya *et al.*, 2010; Zhao *et al.*, 2016) (Cops  
194 *et al.*, 2018a; Cops *et al.*, 2018b). Urine samples were centrifuged (1500 rpm, 5 min) and preserved (-  
195 20°C) for later analysis. Urine samples were analyzed for creatinine, urea, and albumin using an  
196 automated analyzer (Cobas 8000 ISE module and Cobas 8000 c702 and c502 module, Roche  
197 diagnostics, Germany). Urinary kidney injury molecule 1 (KIM-1) concentrations were determined  
198 using the rat TIM-1/KIM-1/HAVCR DuoSet ELISA kit (DY3689, R&D Systems, USA) according to the  
199 manufacturer's instructions and all measurements were performed in duplicate (Cops *et al.*, 2018a;  
200 Cops *et al.*, 2018b). Creatinine clearance was calculated from the following equation: creatinine  
201 clearance (ml/min/kg) = [(urinary creatinine (mg/dl) x urinary volume (ml/24h))/(plasma creatinine  
202 (mg/dl) x 1440 min)]/body weight (kg) (Rafiq *et al.*, 2012).

### 203 *Echocardiography measurements*

204 Echocardiography was performed at baseline and after twelve weeks of exercise training or control  
205 follow-up, under isoflurane anesthesia in spontaneously breathing rats (1.5-2% volume supplemented  
206 with oxygen), using the GE VIVID *i* ultrasound machine and a 10S transducer (GE Vingmed Ultrasound,  
207 version 7.0.1, Norway). A standard parasternal long-axis image and a short-axis image at  
208 midventricular level were acquired using B-mode, at a temporal resolution of  $\approx$  200 frames per second.  
209 Left ventricular end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), posterior and  
210 anterior wall thicknesses (PWT, AWT) were obtained from the parasternal short-axis view. Left  
211 ventricular end-diastolic volume (EDV) and LV end-systolic volume (ESV) were calculated as follows:  $\pi$   
212 \*  $D_M^2$  \* B/6.  $D_M$  indicates the systolic/diastolic diameter of the ventricle on midventricular short-axis  
213 view and B is the LV length on the parasternal long-axis image. Heart rate (HR) was determined by  
214 defining end-systole and end-diastole as the minimum and maximum LV short-axis area, respectively.  
215 Stroke volume (SV) was calculated as EDV – ESV. Cardiac output (CO) was calculated as SV \* HR. LV  
216 fractional shortening (FS) ([LVEDD-LVESD]/LVEDD \* 100) and ejection fraction (EF) ([EDV-ESV]/EDV \*  
217 100) were calculated and expressed in %. Analysis was performed on an EchoPAC workstation (GE  
218 Vingmed Ultrasound, version 7.0.1, Norway) (Cops *et al.*, 2018a; Cops *et al.*, 2018b).

### 219 *Renal Functional Reserve (RFR) experiment*

220 A HPD was administered the week after the end of the treadmill running period (= week 13) for three  
221 consecutive days to stress the kidneys and determine RFR. Prior to administering the HPD (48%

222 protein, 20% fat, 32% carbohydrates, E1509-34, Bio Services, The Netherlands), blood samples were  
223 obtained for measurement of pre-HPD plasma creatinine, sodium and cystatin C levels and urine  
224 samples were collected for the determination of urinary creatinine and sodium excretion. On the third  
225 day of HPD feeding, rats were placed in metabolic cages to obtain 24h food intake and 24h urine  
226 samples, after which blood sampling was repeated. RFR is defined as  $\Delta$  creatinine clearance<sub>week 13</sub> –  
227 creatinine clearance<sub>week 12</sub>. Fractional excretion of sodium (FE<sub>Na</sub>) was calculated as follows: [urinary  
228 sodium (mmol/l) \* plasma creatinine (mg/dl)] / [plasma sodium (mmol/l) \* urinary creatinine (mg/dl)]  
229 (Cops *et al.*, 2018a; Cops *et al.*, 2018b).

### 230 *Hemodynamic measurements*

231 After the RFR experiment, invasive blood pressure measurements were performed under isoflurane  
232 anesthesia (1.5-2% volume supplemented by oxygen) in spontaneously breathing rats. Briefly, a 2F  
233 micro tip high-fidelity pressure catheter (Millar Instruments, AD instruments, Germany), calibrated to  
234 atmospheric pressure before introduction, was first inserted into the right jugular vein, and next into  
235 the left femoral vein and advanced into the abdominal IVC. After stabilization, the central venous  
236 pressure (CVP) was recorded. Finally, the right carotid artery was cannulated and the catheter was  
237 advanced into the left ventricle. After stabilization, LV pressure (LVP) was recorded. Left ventricular  
238 end-diastolic pressure (LVEDP) and the time constant of LV pressure decay during the isovolumic  
239 relaxation period ( $\tau$ ) were calculated using LabChart v7.3.7 software (Millar Instruments, AD  
240 instruments, Germany). Afterwards, rats were sacrificed with an overdose of pentobarbital (200  
241 mg/kg, i.p.) (Cops *et al.*, 2018a; Cops *et al.*, 2018b).

### 242 *Fibrosis measurement*

243 Kidney, liver and heart tissue sections of five  $\mu$ m thick were stained using the Masson trichrome  
244 staining method. Sections were scanned using the Mirax Desk and observed at 20-times magnification  
245 using the Mirax viewer (Carl Zeiss MicroImaging, Germany). Fibrosis was assessed in four randomly  
246 chosen sections in each organ per rat, as described previously (Ichinose *et al.*, 2004; Cops *et al.*, 2018a;  
247 Cops *et al.*, 2018b). The area of collagen deposition was outlined and quantified using an automated  
248 image analysis program (AxioVision 4.6, Carl Zeiss MicroImaging, Germany). Blood vessels were  
249 excluded. Percentage fibrosis was calculated as the ratio of the area of collagen deposition to the  
250 global area (Cops *et al.*, 2018a; Cops *et al.*, 2018b).

### 251 *Kidney morphology*

252 Kidney morphology was assessed in kidney sections subjected to the Masson trichrome staining  
253 method. Glomerular surface area was measured in ten randomly chosen glomeruli per rat and width  
254 of Bowman's space was measured five times per Bowman's space in ten randomly chosen glomeruli

255 per rat, as described previously (Cops *et al.*, 2018a; Cops *et al.*, 2018b). Glomerular density was  
256 calculated by counting well-preserved glomeruli in five randomly selected fields with a surface area of  
257 3.14 mm<sup>2</sup> in renal sections of each rat by using an analysis program (Pannoramic Viewer, 3DHISTECH,  
258 Hungary) (Cops *et al.*, 2018a; Cops *et al.*, 2018b).

#### 259 *Western blot*

260 Protein concentrations of whole kidney samples were determined using the BCA protein assay kit  
261 (Thermo Fisher, Belgium). Samples containing the same amount of proteins were separated on a 12%  
262 SDS-page gel with a mini protean 3 electrophoresis system (Bio-rad Laboratories, Belgium), then  
263 transferred to a polyvinylidene fluoride membrane and blocked 2h with 5% bovine serum albumin  
264 (BSA) in Tris-buffered solution containing 0.1% Tween-20 (TBS-T). To investigate whole kidney RAAS  
265 protein expression, the membrane was incubated overnight at 4°C in the presence of an anti-  
266 angiotensin II type I receptor antibody (ATIIT1R, 1/2000, ab18801, Abcam, UK), as previously described  
267 (Wang *et al.*, 2015). Secondary swine anti-rabbit horseradish peroxidase-conjugated antibody (P0217,  
268 DAKO, Belgium) at a dilution of 1/2500 was used. Both primary and secondary antibodies were diluted  
269 in BSA-TBS-T. ATIIT1R was visualized using the chemiluminescence (ECL) technique (1 min exposure)  
270 using the Pierce ECL Plus Western Blotting Substrate Kit (Thermo Fisher, Belgium) and quantified using  
271 Image Quant TL software v8.1 (GE Healthcare Europe, Belgium). Data were normalized to  $\beta$ -actin  
272 protein levels (1/2500, sc-4778, Santa Cruz, USA) (Cops *et al.*, 2018a; Cops *et al.*, 2018b) (figure S1).

#### 273 *Statistical analysis*

274 Data are expressed as median [25<sup>th</sup> percentile; 75<sup>th</sup> percentile]. After testing for normality using the  
275 Shapiro-Wilk normality test, parameters of IVCC-SED rats were compared to parameters of IVCC-MOD  
276 rats using an unpaired t-test or a Mann-Whitney test as appropriate (GraphPad Prism Software 7.04,  
277 USA). Since this was a pilot experiment, sample size and power calculation could not be conducted. A  
278 two-tailed value of  $P < 0.05$  was considered statistically significant. An observed power  $\geq 0.80$  was  
279 considered sufficient (G\*Power 3.1.9.2, Universität Düsseldorf, Germany) (Cops *et al.*, 2018a; Cops *et*  
280 *al.*, 2018b).

281 **Results**

282 *Baseline comparison*

283 Pre-surgical blood and urinary parameters did not differ between both groups, except for a significantly  
284 higher urinary creatinine excretion ( $P=0.02$ ) in IVCc-MOD rats versus IVCc-SED rats (table 1). Pre-  
285 surgical echocardiographic parameters did also not differ between both groups (table 2).

286 *General effects of exercise intervention*

287 Plasma triglyceride levels ( $P=0.0057$ , table 1), perirenal fat pad/tibia length ratio ( $P=0.0023$ , table 3)  
288 and epididymal fat pad/tibia length ratio ( $P=0.0191$ , table 3) were significantly reduced in IVCc-MOD  
289 rats versus IVCc-SED rats, for a comparable body weight. Plasma lactate levels of IVCc-MOD rats tended  
290 to decrease compared to IVCC-SED rats after twelve weeks of exercise training, although not yet  
291 reaching statistical significance ( $P=0.08$ , fig. 1A). However, plasma lactate levels decreased significantly  
292 in IVCc-MOD rats after twelve weeks of moderate intense endurance exercise training compared to  
293 pre-training levels in IVCc-MOD rats (week 1) ( $P=0.0043$ , fig. 1B). After twelve weeks of exercise  
294 training, body weight gain, kidney weight-, heart weight-, liver weight- and spleen/weight tibia length  
295 ratio did not differ between both groups (table 3).

296 *Abdominal venous pressure was lowered by moderate intense endurance exercise training*

297 After twelve weeks of moderate intense endurance exercise training, jugular venous pressure did not  
298 differ significantly between both groups (0.6 [-0.3;1.6] mmHg in the IVCc group versus 0.2 [-0.9;1.2]  
299 mmHg in the IVCc-SED group,  $P=0.30$ , fig. 2A). In contrast, abdominal venous pressure was significantly  
300 lower in IVCc-MOD compared to IVCc-SED rats (11.6 [9.8;12.3] mmHg versus 13.3 [12.4;14.1] mmHg,  
301 respectively;  $P=0.0148$ ; fig. 2B).

302 *The effect of moderate intense endurance exercise training on kidney function*

303 Plasma creatinine and urea levels were significantly increased in IVCc-MOD rats ( $P=0.0037$  and  
304  $P=0.0113$ , respectively), in contrast to a significantly lower plasma cystatin C level in IVCc-MOD rats  
305 ( $P=0.0041$ ), compared to IVCc-SED rats (table 1), after twelve weeks of moderate intense endurance  
306 training. Urine volume and plasma aldosterone levels tended to increase in IVCc-MOD rats compared  
307 to IVCc-SED rats, although not reaching statistical significance ( $P=0.06$  and  $P=0.08$ , table 1). Creatinine  
308 clearance, water intake, plasma CRP levels and urinary KIM-1, albumin, creatinine excretion and urea  
309 excretion (table 1) did not differ between both groups. Protein expression levels of renal angiotensin  
310 II type I receptor (AT1R), glomerular surface area, width of Bowman's space, glomerular density and  
311 renal collagen deposition did also not differ between both groups (fig. 3A-F).

312 *Proximal tubular integrity was preserved after a high protein loading in exercise-trained rats*

313 Food intake in both groups was similar during the RFR experiment ( $P=0.33$ ). After the oral protein load  
314 (week 13), water intake ( $P=0.03$ ), 24h urine volume ( $P=0.0182$ ) and fractional sodium excretion ( $FE_{Na}$ ,  
315  $P=0.0468$ ) increased significantly in IVCC-MOD rats compared to IVCC-SED rats (table 4). Plasma  
316 creatinine and cystatin C levels did not differ between both groups after the RFR experiment. Fig. 4A  
317 depicts the change in creatinine clearance before and after the HPD for each rat in both experimental  
318 groups. In the IVCC-SED group, creatinine clearance increased in six rats, remained comparable in one  
319 rat and decreased in one rat from week 12 to 13. In the IVCC-MOD group, creatinine clearance  
320 increased in seven rats and decreased in one rat from week 12 to 13. Fig. 4B depicts the RFR ( $\Delta$   
321  $\text{creatinine clearance}_{\text{week 13}} - \text{creatinine clearance}_{\text{week 12}}$ ) but RFR did not differ between both groups.  
322 Urinary KIM-1<sub>post HPD</sub> levels were significantly lower in IVCC-MOD versus IVCC-SED rats ( $P=0.0379$ , table  
323 4).

324 *Hepatic and cardiac function and histology were not affected by moderate intense endurance exercise*  
325 *training*

326 Plasma ALT, AST and bilirubin levels did not differ between both groups (table 1). In general,  
327 conventional echocardiographic parameters and cardiac hemodynamic parameters did not differ  
328 significantly between both groups after twelve weeks of follow-up (table 2). Cardiac and hepatic  
329 collagen deposition did not differ between both groups (fig. 5).

## 330 **Discussion**

331 This study explored the effects of moderate intense endurance exercise training on heart and kidney  
332 function in a rat model with selective abdominal venous congestion, to evaluate whether exercise  
333 intervention is a promising treatment strategy in congestion-related diseases. The main findings are:  
334 (1) twelve weeks of moderate intense endurance exercise training lowers body fat content and  
335 decreases the abdominal venous pressure significantly; (2) exercise training is favorable to kidney  
336 function and (3) cardiac function is largely unaffected by the training intervention. This study is the  
337 first to indicate the benefits of moderate intense endurance exercise training on kidney function in a  
338 rat model of isolated abdominal venous congestion.

### 339 *Exercise training lowers body fat content*

340 In this study, plasma triglyceride levels and perirenal and epididymal fat pads were significantly  
341 reduced in IVCC-MOD rats after twelve weeks of exercise training, while body weight remained  
342 comparable in both groups, indicating a decreased fat mass in moderate exercised rats. Moreover,  
343 exercise training efficacy was evidenced by the decreased longitudinal plasma lactate levels in trained  
344 rats (week 1 versus week 12, fig. 1B) and a trend towards significantly reduced cross-sectional plasma  
345 lactate levels in IVCC-MOD rats versus IVCC-SED rats, after twelve weeks of exercise training (fig. 1A).

### 346 *Moderate intense endurance exercise training is effective to reduce abdominal venous pressure*

347 We previously described an increase in abdominal venous pressure up to 8-18 mmHg sustained over  
348 time, with a mortality rate of respectively 50% and 61% after IVC constriction (Cops *et al.*, 2018a; Cops  
349 *et al.*, 2018b). The mortality rate of the current study (54%) is similar to these previous studies.  
350 Mortality may be reduced in the future by performing a tracheotomy as intubation can lead to upper  
351 airway swelling or obstruction. To further reduce mortality, it was opted to constrict the IVC in young  
352 rats, but young animals may adapt to this constriction (e.g. development of collateral vessels) more  
353 easily than older animals. However, it is important to keep in mind that IVC constriction remains an  
354 invasive procedure and not all rats can cope with the acute effects of IVCC constriction.

355 In the current study, the degree of constriction was visually confirmed after sacrifice and IVCC-MOD  
356 rats demonstrated a significantly decreased median abdominal venous pressure of 11.6 [9.8;12.3]  
357 mmHg versus 13.3 [12.4;14.1] mmHg in IVCC-SED, which is still above the upper limit of normal  
358 (Mullens *et al.*, 2009). To our knowledge, this is the first study to demonstrate the beneficial effects of  
359 moderate intense endurance exercise training on CVP in a rat model of isolated venous congestion.  
360 Venous congestion, irrespective of a reduced cardiac output, is the most important contributor driving  
361 worsening in renal function in heart failure (Mullens *et al.*, 2009; Dupont *et al.*, 2011). Thus, lowering  
362 the CVP diminishes venous congestion and, consequently, worsening in renal function will be delayed.

363 Physical activity improves the blunted excretory response observed after acute volume expansion in  
364 heart failure, as shown in rats with heart failure exposed to an acute sodium loading, by increasing the  
365 vasodilator nitric oxide (NO) and nitric oxide synthase and/or by reducing plasma levels of angiotensin  
366 II (Zheng *et al.*, 2006). In this way, GFR is increased and decongestion is achieved. Indeed, IVCC-MOD  
367 rats of the current study displayed a trend to an increased 24h urine output ( $P=0.06$ ), implying  
368 decongestion in congested rats subjected to exercise training. Second, exercise training can also  
369 promote IVC enlargement, thereby lowering the CVP and reduce congestion, as both the IVC diameter  
370 and the cross-sectional area were significantly increased in young and healthy endurance-trained  
371 females (Hedman *et al.*, 2016). The corresponding IVC venodilation is provoked by NO, CO<sub>2</sub> and  
372 adenosine, to improve blood flow and venous return (S, 2011; Tsukiyama *et al.*, 2017). Moreover, it  
373 has been shown that venodilators are capable to lower the CVP in patients with a Fontan circulation  
374 (Kurishima *et al.*, 2015). Based on the arguments that exercise training promotes release of vaso-  
375 /venodilators and concomitant venodilation, the IVC is dilated in our IVCC-MOD rats and, in this way,  
376 contributing to a reduced CVP. Third, exercise training can promote the development of a collateral  
377 circulation around the IVC constriction, as exercise training has been known to promote coronary  
378 collateralization during coronary artery disease to restore adequate blood flow (Heaps & Parker, 2011).  
379 By circumventing the bottleneck at the site of IVC constriction with collaterals (Kasai *et al.*, 2017), CVP  
380 can also be lowered in our exercised rats. However, this is merely an assumption and could not be  
381 proven in our rat model. Fourth, it was previously demonstrated that abdominal venous congestion  
382 induces systemic inflammation (Cops *et al.*, 2018a) and it is known that aerobic physical activity can  
383 reduce chronic inflammation (Beavers *et al.*, 2010), particularly in chronic diseases. However, no  
384 reduction in plasma CRP levels was observed in IVCC-MOD rats, suggesting a similar inflammatory  
385 status in both experimental groups. In conclusion, all of the above mechanisms may contribute to a  
386 reduced CVP and abdominal venous congestion resulting from exercise training. However, all of these  
387 mechanisms are merely assumptions and the mechanisms by which exercise training reduces CVP have  
388 not yet been established and further research is necessary.

### 389 *Moderate intense endurance exercise is favorable to kidney function in a congestive state*

390 After twelve weeks of exercise training in rats with abdominal venous congestion, kidney function is  
391 better preserved, as indicated by the significantly lower plasma cystatin C levels in trained rats,  
392 compared to sedentary rats. Abdominal venous congestion leads to renal congestion and dysfunction.  
393 As already explained in the study of (Cops *et al.* 2018a), renal congestion leads to tubular compression  
394 and to an augmented luminal pressure, which lowers the transglomerular pressure gradient, thereby  
395 opposing GFR and creating retrogradely transduced glomerular hypertension (Cops *et al.*, 2018b).  
396 When CVP is lowered by exercise training in IVCC-MOD rats, the transglomerular pressure gradient is

397 preserved and both glomerular hypertension and glomerulomegaly are countered. Moreover, exercise  
398 training leads to decongestion in trained rats by improving the renal excretory response, as mentioned  
399 above, thereby relieving the stress exerted on the glomeruli (Zheng *et al.*, 2006). Both mechanisms  
400 may explain the lower cystatin C levels in trained rats. Second, plasma creatinine levels were  
401 significantly increased in IVcC-MOD rats, potentially due to an increased level of precursors of  
402 creatinine, such as creatine phosphate or creatine. Creatine phosphate is required to recycle  
403 adenosine triphosphate (ATP), as the total amount of ATP within the cells is very small and ATP  
404 requirements increase during exercise (Baker *et al.*, 2010). Eventually, creatine phosphate is broken  
405 down into creatinine, thereby accounting for the increased creatinine levels. In addition, aerobic  
406 exercise training has been shown to increase total myocardial creatine kinase activity, the enzyme  
407 responsible for the conversion of creatine to creatine phosphate (Stuewe *et al.*, 2001). As body weight  
408 remains comparable while fat content decreases in IVcC-MOD rats, an increase in muscle mass can  
409 also contribute to a higher serum creatinine. Since the amount of creatinine is determined by muscle  
410 mass, creatinine is not a reliable marker of kidney function in a setting of exercise training. Cystatin C  
411 is a more adequate alternative to establish renal function during and after exercise in this rat model  
412 (Baxmann *et al.*, 2008; Mingels *et al.*, 2009). We could not find a significant difference in levels of  
413 calculated renal creatinine clearance between IVcC and SHAM group, due to large variations in urinary  
414 creatinine excretion levels between individual rats, as reported before (Cops *et al.*, 2018a; Cops *et al.*,  
415 2018b). In this study, there was no evidence of RAAS activity attenuation, systemic inflammation or  
416 increased fibrosis, since plasma aldosterone, protein expression levels of ATIIT1R, plasma CRP, and  
417 renal collagen deposition respectively did not differ between both groups.

418 After cessation of exercise training, RFR was tested with a renal stress test by use of an oral high protein  
419 load, which has been proven to be a safe and feasible tool to quantify RFR in patients (Spinelli *et al.*,  
420 2017). We expected that IVcC-MOD rats were able to increase their RFR by inflation of the glomeruli  
421 and glomerular hyperfiltration, due to the beneficial effect of exercise training on abdominal venous  
422 congestion and kidney function. Contrarily, it was expected that IVcC-SED rats were not able to  
423 increase their RFR to the same extent as trained rats, or not at all, because these rats already  
424 demonstrate glomerulomegaly due to more severe abdominal venous congestion. However, plasma  
425 creatinine, cystatin C and RFR did not differ between both experimental groups after the renal stress  
426 test, suggesting disappearance of the beneficial effect of training on glomerular function after  
427 cessation of physical activity or suggesting that the kidneys of IVcC-SED rats are still capable to increase  
428 their RFR to a similar extent as IVcC-MOD rats. In addition, plasma creatinine levels decreased following  
429 high protein loading in IVcC-MOD rats (table 1 versus table 4). This can be explained by the fact that  
430 exercise training is ceased in week 13. Hence, there is no need for increased ATP requirements  
431 anymore and plasma creatinine levels of IVcC-MOD rats return to similar levels as those observed in

432 IVCc-SED rats. The preservation of RFR at 13 weeks in both sedentary and exercising rats suggests a  
433 potentially reversible renal dysfunction. This assumption is corroborated by the histological data  
434 showing no change in renal morphology in trained rats, after twelve weeks of renal and abdominal  
435 venous congestion. However, exercise training preserved tubular integrity during the oral protein load  
436 challenge, as demonstrated by the significantly lower KIM-1 levels in IVCC-MOD rats. Urinary KIM-1 is  
437 validated and widely used as a biomarker of acute and chronic kidney injury in both rodent models and  
438 patients (Sabbisetti *et al.*, 2014).

439 To summarize, there was no evidence of increased renal fibrosis or altered renal morphology in the  
440 current study, proposing solitary isolated hemodynamically mediated alterations on kidney function.  
441 Hence, even a small reduction in abdominal venous pressure, even above the upper limit of normal of  
442 8 mmHg, by moderate intense endurance exercise training is important to maintain glomerular  
443 function and tubular integrity. The lack of pronounced morphological renal improvements can be  
444 explained by the fact that the abdominal venous pressure is still above the upper limit of normal.

#### 445 *Cardiac and hepatic function are not altered by moderate intense endurance exercise training*

446 Cardiac function was largely unaffected after twelve weeks of exercise training, as indicated by the lack  
447 of changes in cardiac echocardiographic and hemodynamic parameters and a similar heart weight/tibia  
448 length ratio in both experimental groups. Similarly, Kemi *et al.* (2005) did not demonstrate a change in  
449 echocardiographic parameters in healthy female rats after a ten-week treadmill running program to  
450 determine which training modality, moderate versus high intensity, is more beneficial (Kemi *et al.*,  
451 2005). Secondly, CO of both experimental groups was comparable as described in our previous studies,  
452 thereby excluding the effects of a reduced CO on organ function (Cops *et al.*, 2018a; Cops *et al.*, 2018b).  
453 Hence, cardiac function was not compromised by reducing the preload in this IVCC rat model or by  
454 exercise training, making the renal findings even more interesting. Moderate intense endurance  
455 exercise training yielded no improvement in hepatic function, as indicated by comparable plasma  
456 bilirubin, AST and ALT levels and collagen deposition in both experimental groups. The liver is the first  
457 organ affected by the increased abdominal venous pressure and the acute and long-term effects of IVC  
458 constriction are probably too severe. Hence, the potential beneficial isolated effect of exercise training  
459 on hepatic function is lost. Similarly, a recent meta-analysis failed to demonstrate changes in AST and  
460 ALT with exercise training in patients who are overweight or exhibit fatty liver disease, due to several  
461 confounding variables in the included studies and great variation in the exercise training modalities  
462 (Smart *et al.*, 2018).

#### 463 *Limitations*

464 This study had a maximal follow-up of twelve weeks, so only conclusions on the short to middle term  
465 effects of exercise training on abdominal venous congestion can be deferred. In the future, the model  
466 may be investigated for a longer period of time, in a larger cohort to ensure a sufficient power and also  
467 in female rats, to study whether moderate intense endurance exercise training also has a favorable  
468 effect on hepatic and cardiac function. Second, sham groups (sham-SED and sham-MOD) were not  
469 included because the aim of this study was to investigate if exercise training reduces abdominal venous  
470 congestion, thereby delaying kidney dysfunction, as explained in the methods section. Third, renal  
471 blood flow was not assessed. Fourth, evaluation of GFR with an exogenous tracer such as inulin is  
472 lacking. Fifth, exercise training was started in the first week after development of abdominal venous  
473 congestion. Therefore, it is not clear how effective exercise training might be if initiated later in the  
474 disease progression of abdominal venous congestion. Finally, the assessment of cardiac function was  
475 focused on the left-sided heart. However, parameters of right-sided cardiac function may also deviate  
476 as a result of the constriction and should be investigated in the future.

#### 477 **Conclusion**

478 It was demonstrated that moderate intense endurance exercise training significantly decreased  
479 abdominal venous pressure in rats with selective abdominal venous congestion. Importantly, kidney  
480 dysfunction was delayed in rats with selective abdominal venous congestion subjected to exercise  
481 intervention, suggesting that moderate intense endurance exercise is beneficial to kidney function.  
482 Finally, cardiac and hepatic function were not altered by the exercise intervention, implying that  
483 cardiac function is not compromised by reducing the preload in this IVCc rat model or by exercise  
484 training.

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488

489 **Compliance with ethical standards**

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492 **Conflict of interest**

493 Jirka Cops, Bart De Moor, Sibren Haesen, Lien Lijnen, Inez Wens, Lieselotte Lemoine, Carmen Reynders,  
494 Joris Penders, Ivo Lambrichts, Wilfried Mullens and Dominique Hansen all declare that they have no  
495 conflict of interest.

496 **Ethical approval**

497 All applicable international, national and/or institutional guidelines for the care and use of animals  
498 were followed. This study conforms to the EU Directive 2010/63/EU for animal experiments and was  
499 approved by the Ethical Committee for Animal Experiments of Hasselt University, Belgium (protocol  
500 number: 201757).

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666

Table 1: Blood and urinary parameters at baseline and after twelve weeks of moderate intense endurance exercise training in rats with abdominal venous congestion.

	Baseline (week 0)			Week 12		
	IVCc-SED	IVCc-MOD	P-value	IVCc-SED	IVCc-MOD	P-value
Body weight (g)	191 [173;206]	190 [176;198]	0.69	504 [449;564]	516 [507;530]	0.79
Plasma triglycerides (mg/dl) #	82 [50;87]	59 [53;76]	0.51	84 [60;94]	50 [42;60]	0.0057**
Plasma creatinine (mg/dl) #	0.13 [0.11;0.16]	0.13 [0.11;0.14]	0.91	0.30 [0.27;0.31]	0.38 [0.37;0.47]	0.0037**
Plasma urea (mg/dl) #	26 [20;28]	26 [25;29]	0.37	34 [32;37]	42 [38;43]	0.0113*
Plasma cystatin C (mg/dl) #	3.11 [1.87;3.20]	2.34 [1.62;3.00]	0.24	2.87 [2.57;3.06]	1.44 [0.98;2.57]	0.0041**
Creatinine clearance (ml/min/kg)	8.6 [7.2;10.9]	9.3 [7.9;9.8]	0.43	6.8 [5.4;9.9]	7.1 [5.7;7.8]	0.98
Water intake (ml/24h) #	27 [22;23]	22 [21;27]	0.30	27 [23;32]	31 [27;46]	0.13
Urine volume (ml/24h) #	4 [3;5]	3 [3;4]	0.32	12 [11;15]	16 [13;20]	0.06
Urinary KIM-1 (ng/g crea) #	2283 [2212;3836]	2310 [2001;2420]	0.51	588 [414;894]	581 [469;671]	0.53
Urinary albumin (mg/g crea) #	73.9 [50.2;113.0]	83.0 [55.9;136.0]	0.88	43.9 [35.2;62.9]	52.9 [44.7;107.9]	0.20
Urinary creatinine excretion (mg/24h)	63.9 [52.2;89.4]	88.8 [80.3;94.3]	0.02*	131.5 [95.4;162.7]	128.5 [99.6;154.4]	0.80
Urinary urea excretion (mg/24h) #	73924 [68714;82403]	70100 [67614;75798]	0.33	50043 [45479;53996]	50188 [45433;51365]	0.60
Plasma aldosterone (ng/l)				79.6 [37.0;255.0]	234.4 [206.3;296.8]	0.08
Plasma ALT (U/l) #	60.5 [53.5;61.8]	57.2 [50.3;67.0]	0.86	47.5 [40.0;50.5]	55.0 [47.8;62.3]	0.08
Plasma AST U/l) #	109.5 [103.8;113.5]	166.5 [105.0;125.0]	0.25	114.5 [99.0;127.3]	131.5 [111.0;132.8]	0.55
Plasma bilirubin (mg/dl) #	0.3 [0.02;0.04]	0.04 [0.02;0.05]	0.58	0.05 [0.04;0.6]	0.06 [0.04;0.08]	0.61
Plasma CRP (mg/dl) #				0.13 [0.01;0.24]	0.02 [0.00;0.21]	0.56

Data are shown as median [25<sup>th</sup> percentile;75<sup>th</sup> percentile] in IVCc-SED rats (n=8) and IVCc-MOD rats (n=8). Based on the Shapiro-Wilk normality test, data were analyzed using an unpaired t-test

or a Mann-Whitney test (#). \* denotes  $P < 0.05$ , \*\* denotes  $P < 0.01$ . The observed statistical power of inter-group comparisons is 0.65 for baseline urinary excretion and 0.92, 0.81, 0.66 and 0.89

670 for plasma triglycerides, creatinine, urea and cystatin C after twelve weeks, respectively. KIM-1 = kidney injury molecule 1, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CRP  
671 = C-reactive protein.  
672

673 **Table 2: Conventional echocardiography parameters at baseline and after twelve weeks of moderate intense endurance**  
 674 **exercise training in rats with abdominal venous congestion.**

	Baseline (week 0)			Week 12		
	IVCc-SED	IVCc-MOD	P-value	IVCc-SED	IVCc-MOD	P-value
<b>LVEDD (mm)</b>	4.9 [4.1;5.1]	4.6 [4.4;5.0]	0.90	7.4 [6.9;7.7]	7.4 [6.9;7.8]	0.69
<b>LVESD (mm)</b>	3.2 [3;3.3]	3.1 [2.3;3.5]	0.89	3.5 [2.9;4.4]	4.2 [4.0;4.6]	0.04*
<b>PWT (mm)</b>	0.61 [0.55;0.70]	0.62 [0.58;0.66]	0.95	0.71 [0.63;0.73]	0.64 [0.55;0.80]	0.78
<b>AWT (mm)</b>	0.50 [0.48;0.62]	0.60 [0.52;0.70]	0.06	0.68 [0.60;0.75]	0.62 [0.57;0.70]	0.74
<b>HR (bpm)</b>	406 [390;443]	432 [408;175]	0.65	357 [338;386]	359 [331;376]	0.94
<b>EDV (μl)</b>	111 [78;115]	104 [86;127]	0.64	349 [293;394]	338 [318;406]	0.74
<b>ESV (μl)</b>	10 [9;12]	13 [6;17]	0.46	16 [13;28]	24 [20;32]	0.12
<b>CO (ml/min)</b>	37 [29;52]	39 [34;49]	0.72	120 [107;138]	125 [98;133]	0.83
<b>EF (%)</b>	90 [85;83]	91 [84;93]	0.88	95 [91;96]	93 [92;95]	0.26
<b>FS (%)</b>	27 [26;31]	36 [15;44]	0.54	36 [30;42]	35 [33;44]	0.82

675 Data are shown as median [25<sup>th</sup> percentile;75<sup>th</sup> percentile] in IVCc-SED rats (n=8) and IVCc-MOD rats (n=8). Based on the  
 676 Shapiro-Wilk normality test, data were analyzed using an unpaired t-test or a Mann-Whitney test (#). \* denotes  $P < 0.05$ . The  
 677 observed statistical power of inter-group comparisons is 0.56 for LVESD after twelve weeks. LVEDD = left ventricular end-  
 678 diastolic diameter, LVESD = left-ventricular end-systolic diameter, PWT = posterior wall thickness, AWT = anterior wall  
 679 thickness, HR = heart rate, EDV = end-diastolic volume, ESV = end-systolic volume, CO = cardiac output, EF = ejection fraction,  
 680 FS = fractional shortening.

681 **Table 3: Physical and cardiac hemodynamic parameters after twelve weeks of moderate intense endurance exercise**  
 682 **training in rats with abdominal venous congestion.**

Week 12	IVCc-SED	IVCc-MOD	<i>P</i> -value
Body weight gain (g/weeks)	317 [277;352]	328 [318;358]	0.54
Perirenal fat pad weight/tibia length (mg/mm)	165.4 [121.6;200.1]	107.5 [84.3;117.6]	0.001**
Epididymal fat pad weight/tibia length (mg/mm)	147.1 [130.0;173.6]	108.5 [98.9;138.4]	0.02*
Kidney weight/tibia length (mg/mm)	76.8 [65.0;84.3]	83.2 [73.3;93.5]	0.40
Heart weight/tibia length (mg/mm)	27.0 [23.0;31.8]	30.5 [27.3;46.0]	0.58
Liver weight/tibia length (mg/mm)	417.4 [377.4;482.1]	454.4 [421.5;557.2]	0.14
LVP (mmHg)	105.6 [96.4;112.4]	105.2 [102.6;119.4]	0.68
LVEDP (mmHg)	7.9 [4.1;9.2]	5.5 [2.1;15.3]	0.52
Tau (s) <sup>#</sup>	0.010 [0.08;0.012]	0.011 [0.010;0.015]	0.27

683 Data are shown as median [25<sup>th</sup> percentile;75<sup>th</sup> percentile] in IVCc-SED rats (n=8) and IVCc-MOD rats (n=8). Based on the  
 684 Shapiro-Wilk normality test, data were analyzed using an unpaired t-test or a Mann-Whitney test (#). \* denotes *P*<0.05, \*\*  
 685 denotes *P*<0.01. The observed statistical power of inter-group comparisons is 0.93 and 0.70 for perirenal fat pad/tibia length  
 686 ratio and epididymal fat pad weight/tibia length ratio, respectively. LVP = left ventricular pressure, LVEDP = left ventricular  
 687 end-diastolic pressure, tau = time constant of LV pressure decay during the isovolumic relaxation period.

688 **Table 4: Effect of high protein loading on blood and urinary parameters after a twelve-week exercise training period in rats**  
 689 **with abdominal venous congestion.**

<b>Week 13</b>	<b>IVCc-SED</b>	<b>IVCc-MOD</b>	<b>P-value</b>
<b>Food intake (g/24h)</b>	24 [22;24]	26 [20;27]	0.33
<b>Water intake (ml/24h)</b>	44 [34;50]	53 [50;60]	0.03*
<b>Urine volume (ml/24h)</b>	27 [25;33]	34 [30;39]	0.018*
<b>FE<sub>Na</sub></b>	0.20 [0.16;0.25]	0.29 [0.19;0.40]	0.046*
<b>Plasma creatinine (mg/dl) #</b>	0.28 [0.20;0.29]	0.27 [0.25;0.28]	0.99
<b>Plasma cystatin C (mg/dl) #</b>	2.01 [1.05;2.22]	1.70 [0.52;2.37]	0.57
<b>Urinary KIM-1<sub>post HPD</sub> (ng/g creatinine) #</b>	650 [573;824]	514 [417;575]	0.038*

690 Data are shown as median [25<sup>th</sup> percentile;75<sup>th</sup> percentile] in IVCc-SED rats (n=8) and IVCc-MOD rats (n=8). Based on the  
 691 Shapiro-Wilk normality test, data were analyzed using an unpaired t-test or a Mann-Whitney test (#). \* denotes  $P < 0.05$ . The  
 692 observed statistical power of inter-group comparisons is 0.63, 0.70, 0.53 and 0.42 for water intake, urine volume, FE<sub>Na</sub> and  
 693 urinary KIM-1, respectively. FE<sub>Na</sub> = fractional sodium excretion, KIM-1 = kidney injury molecule 1, HPD = high protein diet.

694

695 **Fig 1** Moderate intense endurance exercise training lowers plasma lactate levels after twelve weeks

696 (A) Plasma lactate levels in IVcC-SED rats (n=8) and IVcC-MOD rats (n=8), after twelve weeks of exercise training. (B) Plasma  
697 lactate levels in IVcC-MOD rats (n=8) in week 1 (before start of exercise training) and after 12 weeks. Based on the Shapiro-  
698 Wilk normality test, data were analyzed using a Mann-Whitney test (A) or an unpaired t-test (B). Data are shown as median,  
699 25<sup>th</sup> percentile, 75<sup>th</sup> percentile, minimum and maximum. \*\* denotes  $P < 0.01$ . The observed statistical power of inter-group  
700 comparisons is 0.99 in (B).

701 **Fig 2** Moderate intense endurance exercise training lowers the abdominal venous pressure below the constriction within  
702 twelve weeks

703 Venous pressure measured in (A) the jugular vein and in (B) the abdominal IVC in IVcC-SED rats (n=8) and IVcC-MOD rats  
704 (n=8). Based on the Shapiro-Wilk normality test, data were analyzed using an unpaired t-test (A) or a Mann-Whitney test (B).  
705 Data are shown as median, 25<sup>th</sup> percentile, 75<sup>th</sup> percentile, minimum and maximum. \* denotes  $P < 0.05$ . The observed  
706 statistical power of inter-group comparisons is 0.66 in (B).

707 **Fig 3** Twelve weeks of moderate intense endurance exercise does not affect renal RAAS system activation or morphology in  
708 rats with abdominal venous congestion

709 (A) Representative western blot for whole kidney ATIIT1R and  $\beta$ -actin of IVcC-SED rats (dotted line, n=7) and IVcC-MOD rats  
710 (solid line, n=8). Samples were derived from the same animal experiment and blots were processed in parallel at the same  
711 time. Due to lack of space, samples were divided over two gels. Separation between both gels is indicated by a black dividing  
712 line. After detection of ATIIT1R, blots were stripped to detect  $\beta$ -actin as a loading control. These blots are the original and  
713 unprocessed blots and full-length and uncropped western blots are shown in figure S1. (B) Quantitative analysis of renal  
714 ATIIT1R protein expression normalized to  $\beta$ -actin of IVcC-SED rats (n=7) and IVcC-MOD rats (n=8). (C) Width of Bowman's  
715 space, (D) glomerular surface area, (E) glomerular density and (F) quantification of total collagen from renal sections of IVcC-  
716 SED rats (n=8) and IVcC-MOD rats (n=8). Based on the Shapiro-Wilk normality test, data were analyzed using an unpaired t-  
717 test (C, D, E, F) or a Mann-Whitney test (B). Data are shown as median, 25<sup>th</sup> percentile, 75<sup>th</sup> percentile, minimum and  
718 maximum. ATIIT1R = angiotensin II type I receptor.

719 **Fig 4** RFR was comparable between both groups after the renal stress test provoked by an oral protein load

720 (A) change in creatinine clearance for each individual rat of both the IVcC-SED (n=8) and IVcC-MOD group (n=8) from week  
721 12 (red dot) to week 13 (blue square). (B) RFR of IVcC-SED rats (n=8) and IVcC-MOD rats (n=8). Based on the Shapiro-Wilk  
722 normality test, data were analyzed using a Mann-Whitney test (B). Data are shown as median, 25<sup>th</sup> percentile, 75<sup>th</sup> percentile,  
723 minimum and maximum. RFR = renal functional reserve.

724 **Fig 5** Cardiac and hepatic histology is not affected by moderate intense endurance exercise training

725 (A) Quantification of total collagen from transverse heart sections and (B) quantification of total collagen from transverse  
726 liver sections, both stained with a Masson trichrome staining, of IVcC-SED rats (n=8) and IVcC-MOD rats (n=8), after twelve  
727 weeks of moderate intense endurance exercise training. Based on the Shapiro-Wilk normality test, data were analyzed using  
728 an unpaired t-test. Data are shown as median, 25<sup>th</sup> percentile, 75<sup>th</sup> percentile, minimum and maximum.

729