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

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Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATIIT1R	Angiotensin II type 1 receptor
ATP	Adenosine triphosphate
AWT	Anterior wall thickness
BSA	Bovine serum albumin
CO	Cardiac output
CRP	C-reactive protein
CVP	Central venous pressure
EDV	End-diastolic volume
EF	Ejection fraction
ESV	End-systolic volume
FE_{Na}	Fractional excretion of sodium
G	Gauge (needle diameter)
GFR	Glomerular filtration rate
HPD	High protein diet
HR	Heart rate
IVCc	Inferior vena cava constriction
IVCc-MOD	IVCc rats subjected to moderate intense endurance exercise
IVCc-SED	Sedentary IVCc rats
KIM-1	Kidney injury molecule 1
LVEDD	Left ventricular end-diastolic diameter
LVEDP	Left ventricular end-diastolic pressure
LVESD	Left ventricular end-systolic diameter
LVP	Left ventricular pressure
NO	Nitric oxide
PWT	Posterior wall thickness
RFR	Renal functional reserve
TBS-T	Tris-buffered solution containing 0.1% Tween-20

Abstract

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

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

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

Statement clinical relevance

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

Introduction

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

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

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

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

Methods

Ethical approval and housing

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

Study design

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

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

Experimental protocol

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

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

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

Moderate intense endurance exercise intervention

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

Blood and urine biochemical analysis

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

Echocardiography measurements

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

Renal Functional Reserve (RFR) experiment

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

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

Hemodynamic measurements

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

Fibrosis measurement

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

Kidney morphology

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

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

Western blot

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

Statistical analysis

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

Results

Baseline comparison

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

General effects of exercise intervention

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

Abdominal venous pressure was lowered by moderate intense endurance exercise training

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

The effect of moderate intense endurance exercise training on kidney function

Plasma creatinine and urea levels were significantly increased in IVCc-MOD rats (*P*=0.0037 and *P*=0.0113, respectively), in contrast to a significantly lower plasma cystatin C level in IVCc-MOD rats (*P*=0.0041), compared to IVCc-SED rats (table 1), after twelve weeks of moderate intense endurance training. Urine volume and plasma aldosterone levels tended to increase in IVCc-MOD rats compared to IVCc-SED rats, although not reaching statistical significance (*P*=0.06 and *P*=0.08, table 1). Creatinine clearance, water intake, plasma CRP levels and urinary KIM-1, albumin, creatinine excretion and urea excretion (table 1) did not differ between both groups. Protein expression levels of renal angiotensin II type I receptor (ATIIT1R), glomerular surface area, width of Bowman's space, glomerular density and renal collagen deposition did also not differ between both groups (fig. 3A-F).

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

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

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

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

Discussion

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

Exercise training lowers body fat content

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

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

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

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

Physical activity improves the blunted excretory response observed after acute volume expansion in heart failure, as shown in rats with heart failure exposed to an acute sodium loading, by increasing the vasodilator nitric oxide (NO) and nitric oxide synthase and/or by reducing plasma levels of angiotensin II (Zheng *et al.*, 2006). In this way, GFR is increased and decongestion is achieved. Indeed, IVCc-MOD rats of the current study displayed a trend to an increased 24h urine output (*P*=0.06), implying decongestion in congested rats subjected to exercise training. Second, exercise training can also promote IVC enlargement, thereby lowering the CVP and reduce congestion, as both the IVC diameter and the cross-sectional area were significantly increased in young and healthy endurance-trained females (Hedman *et al.*, 2016). The corresponding IVC venodilation is provoked by NO, CO_2 and adenosine, to improve blood flow and venous return (S, 2011; Tsukiyama *et al.*, 2017). Moreover, it has been shown that venodilators are capable to lower the CVP in patients with a Fontan circulation (Kurishima *et al.*, 2015). Based on the arguments that exercise training promotes release of vaso-/venodilators and concomitant venodilation, the IVC is dilated in our IVCc-MOD rats and, in this way, contributing to a reduced CVP. Third, exercise training can promote the development of a collateral circulation around the IVC constriction, as exercise training has been known to promote coronary collateralization during coronary artery disease to restore adequate blood flow (Heaps & Parker, 2011). By circumventing the bottleneck at the site of IVC constriction with collaterals (Kasai *et al.*, 2017), CVP can also be lowered in our exercised rats. However, this is merely an assumption and could not be proven in our rat model. Fourth, it was previously demonstrated that abdominal venous congestion induces systemic inflammation (Cops *et al.*, 2018a) and it is known that aerobic physical activity can reduce chronic inflammation (Beavers *et al.*, 2010), particularly in chronic diseases. However, no reduction in plasma CRP levels was observed in IVCc-MOD rats, suggesting a similar inflammatory status in both experimental groups. In conclusion, all of the above mechanisms may contribute to a reduced CVP and abdominal venous congestion resulting from exercise training. However, all of these mechanisms are merely assumptions and the mechanisms by which exercise training reduces CVP have not yet been established and further research is necessary.

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

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

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

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

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

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

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

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

Limitations

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

Conclusion

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

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Compliance with ethical standards

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Conflict of interest

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

Ethical approval

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

References

Adams KF, Jr., Fonarow GC, Emerman CL, LeJemtel TH, Costanzo MR, Abraham WT, Berkowitz RL, Galvao M & Horton DP (2005). Characteristics and outcomes of patients hospitalized for heart failure in the United States: rationale, design, and preliminary observations from the first 100,000 cases in the Acute Decompensated Heart Failure National Registry (ADHERE). *American Heart Journal* **149,** 209-216.

Baker JS, McCormick MC & Robergs RA (2010). Interaction among Skeletal Muscle Metabolic Energy Systems during Intense Exercise. *Journal of Nutrition and Metabolism* **2010,** 905612.

Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM & Heilberg IP (2008). Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clinical Journal of the American Society of Nephrology* **3,** 348-354.

Beavers KM, Brinkley TE & Nicklas BJ (2010). Effect of exercise training on chronic inflammation. *Clinica Chimica Acta* **411,** 785-793.

Cao G, González J, Müller A, Ottaviano G, Ambrosio G, Toblli JE & Milei J (2016). Beneficial Effect of Moderate Exercise in Kidney of Rat after Chronic Consumption of Cola Drinks. *PloS One* **11,** e0152461.

Cops J, Haesen S, De Moor B, Mullens W & Hansen D (2019a). Current animal models for the study of congestion in heart failure: an overview. *Heart Failure Reviews*.

Cops J, Haesen S, De Moor B, Mullens W & Hansen D (2019b). Exercise intervention in hospitalized heart failure patients, with emphasis on congestion-related complications: a review. *Heart Failure Reviews*.

Cops J, Mullens W, Verbrugge FH, Swennen Q, De Moor B, Reynders C, Penders J, Achten R, Driessen A, Dendooven A, Rigo J-M & Hansen D (2018a). Selective abdominal venous congestion induces adverse renal and hepatic morphological and functional alterations despite a preserved cardiac function. *Scientific Reports* **8,** 17757.

Cops J, Mullens W, Verbrugge FH, Swennen Q, Reynders C, Penders J, Rigo JM & Hansen D (2018b). Selective abdominal venous congestion to investigate cardiorenal interactions in a rat model. *PloS One* **13,** e0197687.

Damman K, Voors AA, Hillege HL, Navis G, Lechat P, van Veldhuisen DJ & Dargie HJ (2010). Congestion in chronic systolic heart failure is related to renal dysfunction and increased mortality. *European Journal of Heart Failure* **12,** 974-982.

Dupont M, Mullens W & Tang WH (2011). Impact of systemic venous congestion in heart failure. *Current Heart Failure Reports* **8,** 233-241.

Erdem A, Gundogan NU, Usubutun A, Kilinc K, Erdem SR, Kara A & Bozkurt A (2000). The protective effect of taurine against gentamicin-induced acute tubular necrosis in rats. *Nephrology, Dialysis, Transplantation* **15,** 1175-1182.

Forestieri P, Guizilini S, Peres M, Bublitz C, Bolzan DW, Rocco IS, Santos VB, Moreira RS, Breda JR, de Almeida DR, Carvalho AC, Arena R & Gomes WJ (2016). A Cycle Ergometer Exercise Program Improves Exercise Capacity and Inspiratory Muscle Function in Hospitalized Patients Awaiting Heart Transplantation: a Pilot Study. *Braz J Cardiovasc Surg* **31,** 389-395.

Groehs RV, Antunes-Correa LM, Nobre TS, Alves MJ, Rondon MU, Barreto AC & Negrao CE (2016). Muscle electrical stimulation improves neurovascular control and exercise tolerance in hospitalised advanced heart failure patients. *Eur J Prev Cardiol* **23,** 1599-1608.

Heaps CL & Parker JL (2011). Effects of exercise training on coronary collateralization and control of collateral resistance. *J Appl Physiol (1985)* **111,** 587-598.

Hedman K, Nylander E, Henriksson J, Bjarnegard N, Brudin L & Tamas E (2016). Echocardiographic Characterization of the Inferior Vena Cava in Trained and Untrained Females. *Ultrasound in Medicine and Biology* **42,** 2794-2802.

Howden EJ, Fassett RG, Isbel NM & Coombes JS (2012). Exercise training in chronic kidney disease patients. *Sports Medicine* **42,** 473-488.

Ichinose F, Bloch KD, Wu JC, Hataishi R, Aretz HT, Picard MH & Scherrer-Crosbie M (2004). Pressure overload-induced LV hypertrophy and dysfunction in mice are exacerbated by congenital NOS3 deficiency. *American Journal of Physiology: Heart and Circulatory Physiology* **286,** H1070-1075.

Johansen KL (2007). Exercise in the end-stage renal disease population. *Journal of the American Society of Nephrology* **18,** 1845-1854.

Kasai H, Tanabe N, Koshikawa K, Hirasawa Y, Sugiura T, Sakao S & Tatsumi K (2017). The Development of Marked Collateral Circulation due to Inferior Vena Cava Filter Occlusion in a Patient with Chronic Thromboembolic Pulmonary Hypertension Complicated with Anti-phospholipid Syndrome. *Internal Medicine* **56,** 931-936.

Kemi OJ, Haram PM, Loennechen JP, Osnes JB, Skomedal T, Wisloff U & Ellingsen O (2005). Moderate vs. high exercise intensity: differential effects on aerobic fitness, cardiomyocyte contractility, and endothelial function. *Cardiovascular Research* **67,** 161-172.

Kurishima C, Saiki H, Masutani S & Senzaki H (2015). Tailored therapy for aggressive dilatation of systemic veins and arteries may result in improved long-term Fontan circulation. *Journal of Thoracic and Cardiovascular Surgery* **150,** 1367-1370.

Lewinter C, Doherty P, Gale CP, Crouch S, Stirk L, Lewin RJ, LeWinter MM, Ades PA, Kober L & Bland JM (2015). Exercise-based cardiac rehabilitation in patients with heart failure: a meta-analysis of randomised controlled trials between 1999 and 2013. *Eur J Prev Cardiol* **22,** 1504-1512.

Li X & Wang K (2017). Effects of moderate-intensity endurance exercise on angiotensin II and angiotensin II type I receptors in the rat heart. *Mol Med Rep* **16,** 2439-2444.

Martens P & Mullens W (2018). How to tackle congestion in acute heart failure. *Korean Journal of Internal Medicine* **33,** 462-473.

Mingels A, Jacobs L, Kleijnen V, Wodzig W & Dieijen-Visser M (2009). Cystatin C a marker for renal function after exercise. *International Journal of Sports Medicine* **30,** 668-671.

Mullens W, Abrahams Z, Francis GS, Sokos G, Taylor DO, Starling RC, Young JB & Tang WH (2009). Importance of venous congestion for worsening of renal function in advanced decompensated heart failure. *Journal of the American College of Cardiology* **53,** 589-596.

Nijst P & Mullens W (2014). The acute cardiorenal syndrome: burden and mechanisms of disease. *Current Heart Failure Reports* **11,** 453-462.

O'Connor CM, Whellan DJ, Lee KL, Keteyian SJ, Cooper LS, Ellis SJ, Leifer ES, Kraus WE, Kitzman DW, Blumenthal JA, Rendall DS, Miller NH, Fleg JL, Schulman KA, McKelvie RS, Zannad F & Pina IL (2009). Efficacy and safety of exercise training in patients with chronic heart failure: HF-ACTION randomized controlled trial. *JAMA* **301,** 1439-1450.

Rafiq K, Noma T, Fujisawa Y, Ishihara Y, Arai Y, Nabi AH, Suzuki F, Nagai Y, Nakano D, Hitomi H, Kitada K, Urushihara M, Kobori H, Kohno M & Nishiyama A (2012). Renal sympathetic denervation suppresses de novo podocyte injury and albuminuria in rats with aortic regurgitation. *Circulation* **125,** 1402-1413.

S A (2011). Effects of vasodilation and arterial resistance on cardiac output. *Journal of Clinical & Experimental Cardiology* **2**.

Sabbisetti VS, Waikar SS, Antoine DJ, Smiles A, Wang C, Ravisankar A, Ito K, Sharma S, Ramadesikan S, Lee M, Briskin R, De Jager PL, Ngo TT, Radlinski M, Dear JW, Park KB, Betensky R, Krolewski AS & Bonventre JV (2014). Blood kidney injury molecule-1 is a biomarker of acute and chronic kidney injury and predicts progression to ESRD in type I diabetes. *Journal of the American Society of Nephrology* **25,** 2177-2186.

Smart NA, King N, McFarlane JR, Graham PL & Dieberg G (2018). Effect of exercise training on liver function in adults who are overweight or exhibit fatty liver disease: a systematic review and meta-analysis. *British Journal of Sports Medicine* **52,** 834-843.

Spinelli A, Sharma A, Villa G, Samoni S, Ramponi F, Brocca A, Brendolan A, Chiaramonte S, Castellano G, Gesualdo L & Ronco C (2017). Rationale for the Evaluation of Renal Functional Reserve in Living Kidney Donors and Recipients: A Pilot Study. *Nephron* **135,** 268-276.

Stuewe SR, Gwirtz PA & Mallet RT (2001). Exercise training increases creatine kinase capacity in canine myocardium. *Medicine and Science in Sports and Exercise* **33,** 92-98.

Tsukiyama Y, Ito T, Nagaoka K, Eguchi E & Ogino K (2017). Effects of exercise training on nitric oxide, blood pressure and antioxidant enzymes. *Journal of Clinical Biochemistry and Nutrition* **60,** 180-186.

Vaidya VS, Ozer JS, Dieterle F, Collings FB, Ramirez V, Troth S, Muniappa N, Thudium D, Gerhold D, Holder DJ, Bobadilla NA, Marrer E, Perentes E, Cordier A, Vonderscher J, Maurer G, Goering PL, Sistare FD & Bonventre JV (2010). Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. *Nature Biotechnology* **28,** 478-485.

Verbrugge FH, Grieten L & Mullens W (2014). New insights into combinational drug therapy to manage congestion in heart failure. *Current Heart Failure Reports* **11,** 1-9.

Wang J, Lu L, Wang Y, Wu Y, Han J, Wang W, Li C & Tu P (2015). Qishenyiqi Dropping Pill attenuates myocardial fibrosis in rats by inhibiting RAAS-mediated arachidonic acid inflammation. *Journal of Ethnopharmacology* **176,** 375-384.

Wens I, Broekmans T, Hendriks JJA, Savelberg HH, Hesseling MK & BO E (2015). Effects of Exercise Intensity in Experimental Autoimmune Encephalomyelitis. *J Mult Scler* **2**.

Zhao Z, Liao G, Zhou Q, Lv D, Holthfer H & Zou H (2016). Sulforaphane Attenuates Contrast-Induced Nephropathy in Rats via Nrf2/HO-1 Pathway. *Oxidative Medicine and Cellular Longevity* **2016,** 9825623.

Zheng H, Li YF, Zucker IH & Patel KP (2006). Exercise training improves renal excretory responses to acute volume expansion in rats with heart failure. *American Journal of Physiology: Renal Physiology* **291,** F1148-1156.

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 [25th percentile;75th 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

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 = C-reactive protein.

Table 2: Conventional echocardiography 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**
LVEDD (mm)	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
LVESD (mm)	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*
PWT (mm)	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
AWT (mm)	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
HR (bpm)	406 [390;443]	432 [408;175]	0.65	357 [338;386]	359 [331;376]	0.94
EDV (µl)	111 [78;115]	104 [86;127]	0.64	349 [293;394]	338 [318;406]	0.74
ESV (µl)	10 [9;12]	13 [6;17]	0.46	16 [13;28]	24 [20;32]	0.12
CO (ml/min)	37 [29;52]	39 [34;49]	0.72	120 [107;138]	125 [98;133]	0.83
EF (%)	90 [85;83]	91 [84;93]	0.88	95 [91;96]	93 [92;95]	0.26
FS (%)	27 [26;31]	36 [15;44]	0.54	36 [30;42]	35 [33;44]	0.82

Data are shown as median [25th percentile;75th 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 $P<0.05$. The observed statistical power of inter-group comparisons is 0.56 for LVESD after twelve weeks. LVEDD = left ventricular end-diastolic diameter, LVESD = left-ventricular end-systolic diameter, PWT = posterior wall thickness, AWT = anterior wall thickness, HR = heart rate, EDV = end-diastolic volume, ESV = end-systolic volume, CO = cardiac output, EF = ejection fraction, FS = fractional shortening.

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

Week 12	IVCc-SED	IVCc-MOD	*P*-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) #	0.010 [0.08;0.012]	0.011 [0.010;0.015]	0.27

Data are shown as median [25th percentile;75th 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 0.93 and 0.70 for perirenal fat pad/tibia length ratio and epididymal fat pad weight/tibia length ratio, respectively. LVP = left ventricular pressure, LVEDP = left ventricular 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.**

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

690 Data are shown as median [25th percentile;75th 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_{Na} and
693 urinary KIM-1, respectively. FE_{Na} = fractional sodium excretion, KIM-1 = kidney injury molecule 1, HPD = high protein diet.

694

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

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

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

Venous pressure measured in (A) the jugular vein and in (B) the abdominal IVC 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 (A) or a Mann-Whitney test (B). Data are shown as median, 25th percentile, 75th percentile, minimum and maximum. * denotes *P*<0.05. The observed statistical power of inter-group comparisons is 0.66 in (B).

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

(A) Representative western blot for whole kidney ATIIT1R and β-actin of IVCc-SED rats (dotted line, n=7) and IVCc-MOD rats (solid line, n=8). Samples were derived from the same animal experiment and blots were processed in parallel at the same time. Due to lack of space, samples were dived over two gels. Separation between both gels is indicated by a black dividing line. After detection of ATIIT1R, blots were stripped to detect β-actin as a loading control. These blots are the original and unprocessed blots and full-length and uncropped western blots are shown in figure S1. (B) Quantitative analysis of renal ATIIT1R protein expression normalized to β-actin of IVCc-SED rats (n=7) and IVCc-MOD rats (n=8). (C) Width of Bowman's space, (D) glomerular surface area, (E) glomerular density and (F) quantification of total collagen from renal sections of 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 (C, D, E, F) or a Mann-Whitney test (B). Data are shown as median, 25th percentile, 75th percentile, minimum and maximum. ATIIT1R = angiotensin II type I receptor.

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

(A) change in creatinine clearance for each individual rat of both the IVCc-SED (n=8) and IVCc-MOD group (n=8) from week 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 normality test, data were analyzed using a Mann-Whitney test (B). Data are shown as median, 25th percentile, 75th percentile, minimum and maximum. RFR = renal functional reserve.

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

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