

# Serum heart-type fatty acid-binding protein decreases and soluble isoform of suppression of tumorigenicity 2 increases significantly by long-term physical activity

Michael Sponder,<sup>1</sup> Michael Lichtenauer,<sup>2</sup> Bernhard Wernly,<sup>2</sup> Vera Paar,<sup>2</sup> Uta Hoppe,<sup>2</sup> Michael Emich,<sup>3</sup> Monika Fritzer-Szekeres,<sup>4</sup> Brigitte Litschauer,<sup>5</sup> Jeanette Strametz-Juranek<sup>6</sup>

<sup>1</sup>Department of Cardiology, Medical University of Vienna, Vienna, Vienna, Austria

<sup>2</sup>Department of Cardiology, Clinic of Internal Medicine II, Paracelsus Medical University of Salzburg, Salzburg, Austria

<sup>3</sup>Austrian Federal Ministry of Defence and Sports, Austrian Armed Forces, Vienna, Austria

<sup>4</sup>Department of Medical-Chemical Laboratory Analysis, Medical University of Vienna, Vienna, Austria

<sup>5</sup>Department of Pharmacology, Medical University of Vienna, Vienna, Austria

<sup>6</sup>SKA-RZ Bad Tatzmannsdorf, Bad Tatzmannsdorf, Austria

## Correspondence to

Priv.Do. Dr. Michael Sponder, Department of Cardiology, Medical University of Vienna, Vienna 1090, Austria; michael.sponder@meduniwien.ac.at

Accepted 2 December 2018  
Published Online First  
27 December 2018



© American Federation for Medical Research 2019. No commercial re-use. See rights and permissions. Published by BMJ.

**To cite:** Sponder M, Lichtenauer M, Wernly B, et al. *J Invest Med* 2019;**67**:833–840.

## ABSTRACT

The aim of this prospective study was to investigate the influence of long-term physical activity on biomarkers for myocyte ischemia (heart-type fatty acid-binding protein, H-FABP), matrix remodelling/vascular stress (soluble isoform of suppression of tumorigenicity 2, sST2) and inflammation (soluble urokinase-type plasminogen activator receptor, suPAR). In this prospective observational study 109 subjects were recruited, 98 completed the study. Subjects were asked to perform exercise within the calculated training pulse for 8 months. The performance gain was measured/quantified by bicycle stress tests at the beginning and end of the observation period. Twenty-seven subjects with a performance gain <2.9% were excluded. suPAR, H-FABP and sST2 were measured in serum at baseline and after 2, 4 and 8 months by ELISA. We found a significant decrease in H-FABP (1.86 (0.86) to 1.29 (0.98) ng/mL;  $p < 0.01$ ) and a significant increase in sST2 levels (6126 (2759) to 6919 (3720) pg/mL;  $p = 0.045$ ) during the observation period of 8 months while there was no remarkable change in suPAR levels. We interpret the activity-induced decrease in H-FABP as sign of lower subclinical myocardial ischemia and better perfusion, probably due to a more economic metabolism and electrolyte balance. The increase in sST2 might reflect physiological sports-induced vascular stress. As H-FABP and sST2 play an important role in the pathomechanism of ischemic cardiomyopathy (iCMP) further studies should investigate the influence of regular physical activity on these biomarkers in a population of patients with iCMP.

**Trial registration number** NCT02097199.

## INTRODUCTION

Over the last decades, advancements in treatment of cardiovascular disease (CVD) have significantly reduced mortality rates in patients with myocardial infarction (MI), ischemic cardiomyopathy (iCMP) and heart failure. However, CVD remains widely prevalent and it still represents an increasing economic burden

## Significance of this study

### What is already known about this subject?

- Elevated plasma levels of soluble urokinase-type plasminogen activator receptor (suPAR) have been associated with coronary calcification, systemic inflammation and heart failure.
- Heart-type fatty acid-binding protein (H-FABP) is mostly found in the cytoplasm of cardiomyocytes but to a lesser extent also in skeletal muscle heart microvessels and aortic endothelial cells.
- An increase in soluble isoform of suppression of tumorigenicity 2 (sST2) levels has been shown in patients with acute and chronic heart failure, non-ST-elevation myocardial infarction, chronic obstructive pulmonary disease and sepsis.
- These 3 proteins are involved in several physiological but also pathological mechanisms such as inflammation, cardiac and/or vascular stress, fibrosis or cardiac remodelling, occurring in coronary artery disease and heart failure.

### What are the new findings?

- We could show a significant physical activity-mediated decrease in H-FABP and a significant increase in sST2 serum levels, whereas suPAR merely increases during the observation period of 8 months.
- We identified low-density lipoprotein-cholesterol as significant negative predictor for serum H-FABP levels.
- 37% higher sST2 levels in men compared with women.

for most countries of the western world. A broad spectrum of pathophysiological processes such as inflammation, myocardial and hemodynamic stress and apoptosis play a paramount role in the ventricular remodelling. For an earlier diagnosis and better understanding of the disease

## Significance of this study

**How might these results change the focus of research or clinical practice?**

- Decreasing H-FABP levels might be a sign of lower subclinical myocardial ischemia and better perfusion, probably due to a more economic metabolism, better electrolyte balance and vascularization as it occurs in well-trained individuals. Potentially, serum H-FABP might even be used in sports medicine as surrogate for success of long-term training and cardiac adaption in healthy individuals. A physical activity-mediated increase in serum sST2 might be due to vascular stress as it occurs during sports sessions and/or could represent one way of exertion of influence of sports on the immune system by buffering interleukin-33-mediated inflammation.

process, upcoming biomarkers that reflect different cellular pathways in CVD have come into the focus of research. Novel biomarkers could assist to identify patients at risk, optimize treatment and prevention strategies.

The aim of this current study was to analyze new cardiac biomarker levels, that is, suppression of tumorigenicity (ST2), soluble urokinase-type plasminogen activator receptor (suPAR) and heart-type fatty acid-binding protein (H-FABP).

The glycoprotein suPAR is a here-domain membrane-bound receptor, which is mainly expressed by immune cells and to a lesser extent by endothelial and smooth muscle cells.<sup>1</sup> Elevated plasma levels of suPAR have been associated with coronary calcification,<sup>2</sup> systemic inflammation<sup>3</sup> and heart failure.<sup>4</sup>

FABP is a cytoplasmic protein with 15 kDa which is expressed in tissues with active fatty acid metabolism and facilitates long-chain fatty acid transport.<sup>5,6</sup> There exist several different types of FABP depending on the tissue in which they were first found. H-FABP is mostly found in the cytoplasm of cardiomyocytes but to a lesser extent also in skeletal muscle<sup>7</sup> heart microvessels and aortic endothelial cells.<sup>8</sup> The reference values for healthy adults are about between 4.8 and 9.1 µg/L measured in serum.<sup>9,10</sup>

In case of damage, stress and/or death of immune cells interleukin (IL)-33 is secreted and binds to circulating soluble isoform of suppression of tumorigenicity 2 (sST2), which can serve as a kind of decoy receptor for IL-33 inhibiting the formation of the IL-33/ST2-ligand (ST2L) complex and, finally, on the one hand attenuating IL-33-mediated inflammation,<sup>11</sup> but on the other hand limiting the cardioprotective effect of IL-33/ST2L activation. An increase in sST2 levels has been shown in patients with acute and chronic heart failure,<sup>12,13</sup> non-ST-elevation myocardial infarction (NSTEMI),<sup>14</sup> chronic obstructive pulmonary disease (COPD) and sepsis.<sup>15</sup>

These 3 proteins are involved in several physiological but also pathological mechanisms such as inflammation, cardiac and/or vascular stress, fibrosis or cardiac remodelling, occurring in coronary artery disease (CAD) and heart failure. As regular physical activity has been shown to have numerous beneficial effects on the cardiovascular system, in particular

concerning inflammation<sup>16,17</sup> or the lipid profile,<sup>18,19</sup> it was the aim of this prospective study to investigate the influence of long-term physical activity on suPAR, sST2 and H-FABP.

**MATERIALS AND METHODS**

In total, 109 subjects were recruited. The inclusion criteria were: age 30–65 years and the physical ability to perform a bicycle stress test and endurance training. Exclusion criteria were: age <30 or >65 years, no ability to perform a bicycle stress test and endurance exercise, current infectious and/or oncologic disease (anamnesic or increased baseline inflammation parameters). Of 109 subjects, 11 did not complete the study for different reasons, for example, accidents, loss of motivation, and so on. Ninety-eight subjects completed the study but 27 subjects did not increase their performance by at least 2.9% or more and therefore were excluded; however, their data concerning the mentioned biomarkers are presented anyway. Finally, the study population was composed of 71 subjects with a proven performance gain over 8 months of observation period and with at least 1 classic cardiovascular risk factor defined as follows: overweight (body mass index (BMI) >25.0 kg/m<sup>2</sup>), hypertension (systolic blood pressure (SBP) >140 ± diastolic blood pressure (DBP) >85 mm Hg at rest/antihypertensive medication), hyper/dyslipidemia (anamnesic therapy with statins), diabetes mellitus (HbA1c >6.5 rel%/antidiabetic medication), current smoking, known chronic heart disease (anamnesic MI, percutaneous coronary intervention, coronary artery bypass graft, stroke) and a positive family anamnesis for MI/CVD/stroke of mother and/or father. The weekly alcohol intake was measured/quantified by units: 1 unit corresponds to 0.33 mL beer, 0.125 mL red/white wine or 0.02 mL spirits.

The study was carried out in adherence to the Declaration of Helsinki and its later amendments. Informed consent was obtained from all subjects before inclusion.

**Measurement of anthropometric data and bicycle stress test**

After anamnesis and physical examination including anthropometric data (height, weight, body water/muscle mass/fat with a diagnostic scale, Beurer BG 16, Beurer, Ulm, Germany), the subjects had to do a bicycle stress test at the beginning of the study to define the individual performance level and to calculate an individual training pulse and target heart rate (using the Karvonen formula with an intensity level of 65%–75% for moderate intensity and 76%–93% for vigorous intensity). The subjects decide for themselves the kind of activity/sports; however, they were asked to do sports for at least 75 min/wk of vigorous intensity or 150 min/wk of moderate intensity (or a mixture; strength training was also allowed but not mandatory) within the previously calculated training pulse. A second bicycle stress test was done after 8 months of training at the end of the study to prove and quantify exactly and objectively the performance change/gain. The bicycle stress tests were always performed with the same system (Ergometer eBike comfort, GE Medical Systems, Freiburg, Germany) and protocol starting with a resistance of 25 W and increasing the resistance every 2 minutes by 25 W (according to the protocol of the Austrian Society of

Cardiology which is equal to the guidelines of the European Society of Cardiology). SBP and DBP and heart rate were taken every 2 minutes, the subjects were permanently ECG monitored. The subjects were told to cycle with 50–70 revolutions/minute until exhaustion occurred. The target performance was calculated using sex, age and body surface (calculated according to DuBois formula: body surface ( $\text{m}^2$ ) =  $0.007184 \times \text{height (cm)}^{0.725} \times \text{weight (kg)}^{0.425}$ ).<sup>20</sup> A target performance of 100% represents the performance of an untrained collective. Concerning nutrition, the subjects were requested not to change their eating habits. However, the subjects also obtained a training diary to record their training effort during the study period.

### Laboratory analysis

Blood samples were drawn in a not starving state. Blood samples for the determination of routine laboratory parameters and suPAR, H-FABP and sST2 were taken at baseline, after 2, 4 and 8 months. All blood samples were taken after 10 minutes of still lying from an arm vein with a tube/adaptor system. Samples for the determination of routine laboratory parameters were analyzed immediately after drawing before the bicycle stress tests.

### ELISA analysis of cardiac biomarkers

Serum levels of sST2, H-FABP and suPAR were analyzed by using ELISA kits that are commercially available (DuoSet DY206, DY1678, DY807; R&D Systems, USA). Preparation of all necessary reagents and measurements was performed according to the instructions supplied by the manufacturer. In short, patient's serum samples and standard protein were added to the wells of the ELISA plates (Nunc MaxiSorp flat-bottom 96-well plates, VWR International, Austria) and were incubated for 2 hours. ELISA plates were then washed using a Tween 20/phosphate buffered saline solution (Sigma Aldrich, USA). In the next step, a biotin-labelled antibody was added and plates were incubated for another 2 hours. Plates were then washed once more and a streptavidin-horseradish-peroxidase solution was added to the wells. By adding tetramethylbenzidine (Sigma Aldrich) a colour reaction was generated. Optical density values were measured at 450 nm on an ELISA plate reader (iMark Microplate Absorbance Reader, Bio-Rad Laboratories, Austria).

The analysis was performed according to the manufacturer's instructions. The coefficients of variation were: for suPAR 2.1%–7.5% intra-assay and 5.6%–5.9% inter-assay, for H-FABP 0.3%–4.7% intra-assay and 1.3%–17.4% interassay and for sST2 4.5%–5.6% intra-assay and 6.3%–7.1% interassay.

### Statistical analysis

Statistical analysis was accomplished using SPSS V.20.0. Continuous and normally distributed data are described by mean  $\pm$  SD. Non-normally distributed data are described by median/25th quartile/75th quartile. Single correlations involving only 2 normally distributed data were calculated using Pearson correlation, single correlations involving 2 non-parametric data and/or ordinal data were calculated using Spearman's rho analysis. Backwards multiple linear regression analysis was performed to investigate the

association of covariables which correlated significantly with baseline suPAR, H-FABP and sST2 levels. To investigate the progression of the parameters of interest over the observation period we used the Friedman test. All tests were performed in accordance with two-sided testing and p values  $\leq 0.05$  were considered significant.

### RESULTS

Risk factor profile, anthropometric data and the results of routine laboratory analysis at baseline as well as the correlation of routine laboratory analysis at baseline with suPAR, H-FABP and sST2 (of 98 subjects who completed the study) are shown in [table 1](#). Seventy-one subjects (45 men, 26 women) had a performance gain of at least 2.9%. The mean performance gain was  $12.1\% \pm 6.3\%$ . The most prevalent cardiovascular risk factors were overweight/adipositas (66%), ex-smoking (42%), familial predisposition (41%), hypertension (32%) and dyslipidemia (27%). According to the training diaries, the cohort had a mean amount of moderate intensity training of  $1248 \pm 940$  min/mo and of vigorous intensity training of  $298 \pm 352$  min/wk. A sex-specific analysis showed that male subjects had significantly higher baseline levels of sST2 compared with female subjects ( $4805 \pm 2629$  vs  $7569 \pm 2443$  pg/mL;  $p < 0.001$ ) but there was no difference concerning suPAR ( $1727 \pm 501$  vs  $1787 \pm 608$  pg/mL;  $p = 0.600$ ) and H-FABP ( $2.01 \pm 1.06$  vs  $1.92 \pm 0.81$  ng/mL;  $p = 0.645$ ).

The correlations of suPAR, H-FABP and sST2 with anthropometric data and routine laboratory analysis are also shown in [table 1](#). The 3 parameters correlated with numerous anthropometric and routine laboratory parameters. Thus, to identify significant predictors of suPAR, H-FABP and sST2 at baseline we performed a backwards multiple linear regression analysis with those parameters which showed a significant Spearman correlation with suPAR, H-FABP and/or sST2. Significant negative predictors for suPAR were potassium, for H-FABP low-density lipoprotein (LDL)-cholesterol and for sST2 body muscle mass. Significant positive predictors for suPAR were age, for H-FABP apolipoprotein B and uric acid and for sST2 male sex and alkaline phosphatase ([table 2](#)).

As expected, we found significant changes in body composition and hemodynamic parameters over the observation period: while BMI ( $27.6 \pm 4.3$  vs  $27.2 \pm 3.8$  kg/m<sup>2</sup>;  $p = 0.319$ ), body muscle mass ( $35.1 \pm 4.2$  vs  $35.3\% \pm 4.1\%$ ;  $p = 0.150$ ) and SBP ( $132 \pm 11$  vs  $131 \pm 11$  mm Hg;  $p = 0.591$ ) did not change significantly, body water percentage ( $52.4 \pm 5.8$  vs  $54.7\% \pm 6.1\%$ ;  $p < 0.001$ ), body fat percentage ( $29.5 \pm 9.9$  vs  $25.9\% \pm 8.4\%$ ;  $p < 0.001$ ) and DBP ( $78 \pm 7$  vs  $75 \pm 7$  mm Hg;  $p = 0.008$ ) decreased significantly.

As shown in [table 3](#) and [figure 1](#), suPAR slightly increased during the observation period from  $1794 \pm 515$  to  $1839 \pm 570$  pg/mL but without statistical significance ( $p = 0.406$ ). However, H-FABP levels decreased significantly from  $1.86 \pm 0.86$  to  $1.29 \pm 0.98$  ng/mL ( $p < 0.001$ ) and sST2 levels increased from  $6126 \pm 2759$  to  $6919 \pm 3720$  pg/mL ( $p = 0.045$ ).

Concerning the excluded subjects, we found no significant progression over the observation period (suPAR:  $1668 \pm 637$  vs  $1678 \pm 504$  pg/mL,  $p = 0.770$ ; H-FABP:

**Table 1** Baseline risk factor profile, anthropometric, routine laboratory parameters and correlations

	n/% of population/ mean±SD	suPAR (pg/mL)	H-FABP (ng/mL)	sST2 (pg/mL)
Hypertension	23/32.4			
Dyslipidemia	19/26.8			
Diabetes mellitus	1/1.4			
Overweight/adipositas	46/65.8			
Ex-smoking	30/42.3			
Smoking	12/16.9			
Known CHD/stroke	14/19.7			
Positive family anamnesis	29/40.8			
Alcohol (units/wk)	3.0±3.9	0.006/0.955	0.052/0.619	0.177/0.092
Age (y)	48.9±6.9	0.216/0.032	−0.018/0.858	0.065/0.529
BMI (kg/m <sup>2</sup> )	27.6±4.3	−0.079/0.440	0.259/0.010	0.214/0.036
Body water (%)	52.4±5.8	0.008/0.940	−0.279/0.005	0.148/0.150
Body fat (%)	29.5±9.9	0.013/0.901	0.274/0.006	−0.193/0.059
Body muscle (%)	35.1±4.2	0.016/0.874	−0.052/0.608	0.337/0.001
SBP (mm Hg)	132±11	0.061/0.552	0.106/0.302	0.167/0.105
DBP (mm Hg)	78±7	0.024/0.818	0.039/0.708	0.142/0.171
Performance 1 (%)	103.8±18.6	−0.060/0.555	−0.280/0.005	0.104/0.312
Performance 2 (%)	115.9±19.0	−0.013/0.900	−0.319/0.001	0.028/0.788
Performance gain (%)	12.1±6.3	0.026/0.810	−0.074/0.469	−0.099/0.336
Moderate intensity (min/mo)	1248±940	0.052/0.637	−0.043/0.694	−0.052/0.641
Vigorous intensity (min/mo)	298±352	−0.058/0.596	−0.067/0.542	<0.001/0.994
Erythrocytes (x10 <sup>12</sup> /L)	4.8±0.4	0.033/0.748	0.204/0.045	0.322/0.001
Haemoglobin (g/L)	142±13	0.018/0.861	0.237/0.020	0.403/<0.001
Haematocrit (%)	40.1±3.3	0.020/0.847	0.231/0.023	0.394/<0.001
Thrombocytes (x10 <sup>9</sup> /L)	243±51	−0.030/0.771	0.002/0.984	−0.160/0.121
Leukocytes (x10 <sup>9</sup> /L)	6.4±1.5	−0.111/0.279	0.076/0.459	0.027/0.797
Na (mmol/L)	141±2	0.001/0.996	−0.222/0.029	0.122/0.240
K (mmol/L)	4.2±0.3	−0.205/0.044	−0.167/0.102	−0.162/0.116
Cl (mmol/L)	101±2	−0.093/0.365	−0.205/0.044	−0.001/0.992
Ca (mmol/L)	2.3±0.1	0.101/0.324	0.138/0.177	0.079/0.448
Phosphate (mmol/L)	1.1±0.1	−0.151/0.139	−0.238/0.019	−0.017/0.868
Mg (mmol/L)	0.85±0.06	−0.102/0.321	0.087/0.398	0.080/0.442
Creatinine (mg/dL)	0.9±0.2	0.103/0.316	0.161/0.115	0.417/<0.001
BUN (mg/dL)	16±17	0.066/0.522	0.054/0.602	0.244/0.017
Uric acid (md/dL)	5.2±1.5	0.021/0.836	0.223/0.028	0.338/0.001
Lipase (U/L)	40±17	−0.014/0.892	−0.066/0.518	−0.018/0.861
Cholinesterase (kU/L)	8.3±1.7	−0.131/0.200	0.256/0.011	0.357/<0.001
Alkaline phosphatase (U/L)	64±41	−0.111/0.279	0.124/0.227	0.229/0.025
ASAT (U/L)	25±8	−0.009/0.928	0.177/0.083	0.432/<0.001
ALAT (U/L)	27±12	−0.089/0.388	0.162/0.113	0.494/<0.001
Gamma-GT (U/L)	31±49	0.057/0.581	0.298/0.003	0.317/0.002
LDH (U/L)	173±25	0.059/0.564	0.196/0.054	0.106/0.309
Triglycerides (mg/dL)	133±82	0.089/0.386	0.356/<0.001	0.146/0.157
Cholesterol (mg/dL)	201±37	0.065/0.527	0.209/0.040	0.005/0.960
HDL-cholesterol (mg/dL)	58±18	−0.041/0.690	−0.210/0.039	−0.206/0.045
LDL-cholesterol (mg/dL)	117±34	0.066/0.522	0.243/0.017	0.053/0.609
Apolipoprotein A1 (mg/dL)	156±29	0.015/0.885	−0.054/0.601	−0.191/0.064
Apolipoprotein B (mg/dL)	103±26	0.089/0.387	0.355/<0.001	0.078/0.451
proBNP (pg/mL)	44±42	0.084/0.412	0.008/0.937	−0.108/0.298
HbA <sub>1c</sub> (rel%)	5.3±0.6	0.119/0.246	0.029/0.781	−0.014/0.889
hsCRP (mg/dL)	0.19±0.18	0.202/0.048	0.275/0.006	0.040/0.702
IL-6 (pg/mL)	2.6±1.6	0.047/0.646	0.166/0.104	0.098/0.343

The table shows first, the cardiovascular risk factor profile, anthropometric data and lab analysis at baseline of the subjects who had a performance gain >2.9% and second, the correlation coefficient and significance of the correlation between suPAR, H-FABP and sST2 with anthropometric and routine laboratory parameters of all subjects who completed the study.

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BMI, body mass index; BNP, brain natriuretic peptide; BUN, blood urea nitrogen; Cl, chloride; Ca, calcium; CHD, chronic heart disease; DBP, diastolic blood pressure; GT, glutamyl transferase; HDL, high-density lipoprotein; H-FABP, heart-type fatty acid-binding protein; HR, heart rate; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; K, potassium; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; Mg, magnesium; Na, sodium; SBP, systolic blood pressure; sST2, soluble isoform of suppression of tumorigenicity 2; suPAR, soluble urokinase-type plasminogen activator receptor.



**Table 2** Results from the backwards multiple linear regression analysis

		Regression coefficient B	SE	$\beta$	T	Significance
suPAR	Constant	2 826 297	989 168		2.857	0.006
	Age	21.702	8.268	0.296	2.625	0.011
	Potassium	-507.487	223.642	-0.256	-2.269	0.026
$F=5.6$ ; $p<0.01$ ; $r^2_{adj}$ : 0.116						
sST2	Constant	5982.284	3471.769		1.723	0.090
	Sex	3986.780	1272.989	0.713	3.132	0.003
	Body muscle	-319.276	137.530	-0.490	-2.322	0.023
	Alkaline phosphatase	17.703	6.755	0.273	2.621	0.011
	Creatinine	4307.157	2440.313	0.249	1.765	0.082
$F=8.96$ ; $p<0.001$ ; $r^2_{adj}$ : 0.319						
H-FABP	Constant	14.560	7.552		1.928	0.058
	Uric acid	0.131	0.062	0.228	2.117	0.038
	LDL-C	-0.017	0.008	-0.691	-2.249	0.028
	Apolipoprotein B	0.029	0.010	0.896	2.811	0.007
$F=7.7$ ; $p<0.001$ ; $r^2_{adj}$ : 0.324						

Significant predictors for suPAR, sST2 and H-FABP according to the backwards multiple linear regression analysis.

H-FABP, heart-type fatty acid-binding protein; LDL-C, low-density lipoprotein-cholesterol; sST2, soluble isoform of suppression of tumorigenicity 2; suPAR, soluble urokinase-type plasminogen activator receptor.

$2.27 \pm 1.24$  vs  $1.73 \pm 1.13$  ng/mL,  $p=0.115$ ; sST2:  $6995 \pm 3076$  vs  $7472 \pm 3503$  pg/mL,  $p=0.101$ ).

## DISCUSSION

In this prospective study, which is the first one to investigate the influence of physical activity on suPAR, H-FABP and sST2, we could show a significant physical activity-mediated decrease in H-FABP and a significant increase in sST2

serum levels, whereas suPAR merely increases during the observation period of 8 months.

H-FABP is involved in the modulation of the cardiac energy production by controlling the transfer of acetyl-L-carnitine to the mitochondrial beta-oxidative system. It has been suggested that H-FABP might act as protective molecule against the toxic effect of (too) high intracellular levels of fatty acids (eg, occurring during ischemia).<sup>21</sup> In mice lacking H-FABP, the heart is unable to efficiently take up plasma-free long-chain fatty acids and use glucose instead leading to intolerance of acute exercise and later, resulting in asymmetrical septal hypertrophy.<sup>22</sup> Therefore, the presence of H-FABP in the cardiomyocytes is absolutely necessary for a high flux of long-chain fatty acids. However, H-FABP is released during myocardial ischemia, damage and necrosis, for example, in course of myocardial ischemia.<sup>6</sup> H-FABP then leaks to extracellular space and enters circulation. It has been shown that H-FABP levels raise within 1–3 hours after acute MI, reach a peak around 4 hours and decline to baseline levels after 24 hours.<sup>23</sup> However, elevated H-FABP levels were also associated with adverse long-term outcome (cardiac death, recurrent MI) after NSTEMI independent of clinical risk profile (Global Registry of Acute Coronary Events [GRACE] score), troponin T (TnT), brain natriuretic peptide and high-sensitivity C-reactive protein (hsCRP) levels.<sup>24</sup> Similar to TnT, H-FABP is also elevated in patients with chronic renal dysfunction and tachyarrhythmia.<sup>25</sup>

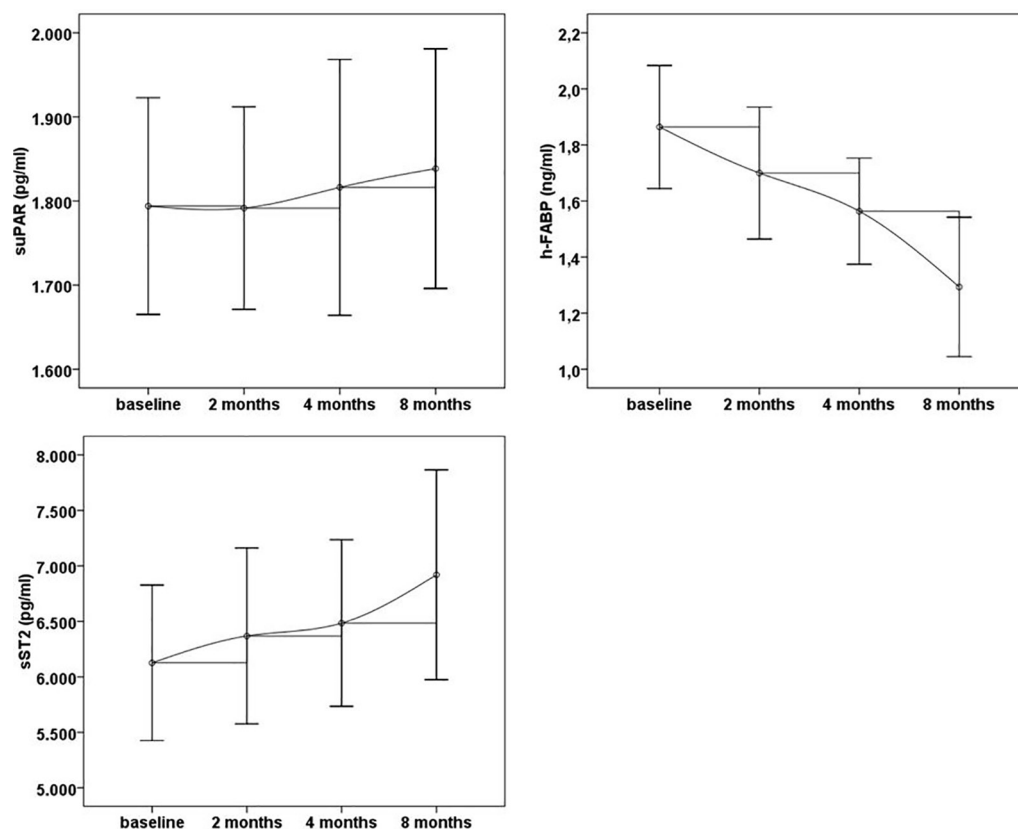
The availability of data concerning the relationship between FABP and sports is very limited. There exists only 1 study which showed an increase in plasma FABP (not H-FABP) after acute exercise due to muscle injury.<sup>26</sup> Concerning H-FABP, Arı *et al* could show that H-FABP levels do not increase due to myocardial ischemia induced by (acute) exercise stress testing in patients with CAD<sup>27</sup> and similar results were found by Sbarouni *et al*.<sup>28</sup> A further study in patients with end-stage renal disease and haemodialysis showed that intradialytic exercise (5-minute warm-up

**Table 3** Levels of suPAR, H-FABP and sST2 during the observation period

	mean $\pm$ SD
suPAR baseline	1794 $\pm$ 515 pg/mL
suPAR 2 mo	1792 $\pm$ 482 pg/mL
suPAR 4 mo	1816 $\pm$ 609 pg/mL
suPAR 8 mo	1839 $\pm$ 570 pg/mL
P value	0.406
$\chi^2$	2.906
H-FABP baseline	1.86 $\pm$ 0.86 ng/mL
H-FABP 2 mo	1.70 $\pm$ 0.93 ng/mL
H-FABP 4 mo	1.56 $\pm$ 0.74 ng/mL
H-FABP 8 mo	1.29 $\pm$ 0.98 ng/mL
P value	<0.001
$\chi^2$	19.124
sST2 baseline	6126 $\pm$ 2759 pg/mL
sST2 2 mo	6368 $\pm$ 3118 pg/mL
sST2 4 mo	6484 $\pm$ 2955 pg/mL
sST2 8 mo	6919 $\pm$ 3720 pg/mL
P value	0.045
$\chi^2$	8.052

Levels of suPAR, H-FABP and sST2 at the different points of measurement and p value of the Friedman test.

H-FABP, heart-type fatty acid-binding protein; sST2, soluble isoform of suppression of tumorigenicity 2; suPAR, soluble urokinase-type plasminogen activator receptor.



**Figure 1** Progression of soluble urokinase-type plasminogen activator receptor (suPAR), heart-type fatty acid-binding protein (H-FABP) and soluble isoform of suppression of tumorigenicity 2 (sST2) over the observation period.

followed by a 30-minute bout of intradialytic exercise using a specially designed cycle ergometer) had no significant influence on H-FABP levels.<sup>29</sup> A small study investigated the influence of a 21km run in 10 healthy male subjects and found a significant increase in H-FABP levels after the run and H-FABP levels returned to baseline 6 hours after the run.<sup>30</sup> In the present study, we could show long-term physical activity-mediated decrease in H-FABP levels. As H-FABP leaks to extracellular space and enters circulation in case of impaired myocardial perfusion we hypothesize that the decreasing H-FABP levels are a sign of lower subclinical myocardial ischemia and better perfusion, probably due to a more economic metabolism, better electrolyte balance and vascularization as it occurs in well-trained individuals. Following this train of thought the measurement of serum H-FABP amounts might even serve as surrogate or follow-up parameter for training success, for example, in patients suffering from iCMP but also healthy individuals. However, as an acute bout of exercise leads to H-FABP release into circulation caused by ‘physiological’ myocardial damage there should be enough time (at least 24 hours) between the last training session and the H-FABP measurement to avoid ‘false positive’ results. In addition, we identified LDL-cholesterol as significant negative predictor for serum H-FABP levels.

Soluble ST2, a member of the IL-1 receptor family, has first been identified in 1989.<sup>31</sup> Recent works have shown that the predominant source of sST2 in human is the vascular endothelium rather than myocardium.<sup>32,33</sup> Although there

have not been defined official reference levels yet, the concentration in healthy individuals is commonly below 25–50 ng/mL in serum and plasma where different sST2 assays are not comparable.<sup>11</sup> However, in general, men show slightly higher levels compared with female individuals.<sup>34,35</sup> This sex-specific difference could be confirmed by our results. In our cohort, men showed about 37% higher sST2 levels compared with females. Several studies stated an increase in sST2 levels in patients with acute and chronic heart failure,<sup>12,13</sup> NSTEMI,<sup>14</sup> COPD and sepsis.<sup>15</sup> However, sST2 levels seem to be associated with inflammation parameters and a worse outcome in above-mentioned diseases, in particular with all-cause and cardiovascular mortality, but it is not associated with traditional risk factors or non-fatal cardiac events. The Dallas Heart Study (n=3294) could also show significant correlations between plasma sST2 levels and growth differentiation factor 15, osteoprotegerin and pulmonary surfactant protein B, and negative correlations with soluble receptor for advanced glycation end products, hsCRP.<sup>36</sup>

In case of damage, stress and/or death of immune cells IL-33 is secreted and binds to ST2L, a membrane bound receptor leading to transcription of inflammatory genes and finally to the production of inflammatory cytokines and chemokines. However, it has also been described that the IL-33/ST2L signalling reduces cardiac hypertrophy and prevents cardiac fibrosis and apoptosis after ischemic injury.<sup>37,38</sup> In case IL-33 binds to circulating sST2, sST2 can serve as a kind of decoy receptor for IL-33 inhibiting the

formation of the IL-33/ST2L complex and, finally, on the one hand attenuating IL-33-mediated inflammation,<sup>11</sup> but on the other hand limiting the cardioprotective effect of IL-33/ST2L activation. An increase in IL-33 serum levels due to physical activity (12 weeks of aerobic resistance training) has already been described in patients suffering from diabetes.<sup>39</sup> In this study, we stated a significant physical activity-mediated increase in sST2 serum levels. We speculate that this increase might be explained by 2 mechanisms: First, physical activity leads to increased blood pressure and physiological vascular stress for the period of the training session and might therefore be causal for an increase in sST2. Second, as sST2 acts as decoy receptor for IL-33 the sST2 increase might buffer IL-33-mediated inflammation.

Concerning suPAR, we only stated a minimal increase without statistical significance. suPAR is expressed by immune cells and to a lesser extent by endothelial and smooth muscle cells. Therefore, the slight increase might be interpreted as vascular reaction due to physical activity; however, we doubt a clinical relevance of this minimal suPAR increase.

## CONCLUSION

Decreasing H-FABP levels might be a sign of lower subclinical myocardial ischemia and better perfusion, probably due to a more economic metabolism, better electrolyte balance and vascularization as it occurs in well-trained individuals. As H-FABP and sST2 play an important role in the pathomechanism of iCMP further studies should investigate the influence of regular physical activity on these biomarkers in a population of patients with iCMP. Potentially, serum H-FABP might even be used in sports medicine as surrogate for success of long-term training and cardiac adaption in healthy individuals. In addition, we identified LDL-cholesterol as significant negative predictor for serum H-FABP levels. A physical activity-mediated increase in serum sST2 might be due to vascular stress as it occurs during sports sessions and/or could represent one way of exertion of influence of sports on the immune system by buffering IL-33-mediated inflammation. Furthermore, we found about 37% higher sST2 levels in men compared with women at baseline.

## Limitations

First, several other not controlled influences and circumstances (besides physical activity) might have had an influence on the analyzed parameters. Second, due to the exclusion of subjects with a performance gain <2.9% the cohort consists of only 1 'intervention' group and no control group although data of the excluded subjects are presented. Third, the population was too small to deliver a sex-specific analysis.

**Contributors** MS: study design, clinical investigation, performing bicycle stress tests/ follow-up, statistical analysis, manuscript preparation. ML and VP: laboratory analysis, manuscript preparation. BW: statistical analysis, laboratory analysis. UH: infrastructure support, manuscript preparation. ME: study design. MFS: laboratory analysis. BL: statistical analysis. JSJ: study design, manuscript preparation.

**Funding** The study has been funded by means of the Austrian Federal Ministry of Defence and Sports, Vienna, Austria

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Ethics approval** Ethics Committee of the Medical University of Vienna (EC No: 1830/2013).

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

- Smith HW, Marshall CJ. Regulation of cell signalling by uPAR. *Nat Rev Mol Cell Biol* 2010;11:23–36.
- Sørensen MH, Gerke O, Eugen-Olsen J, et al. Soluble urokinase plasminogen activator receptor is in contrast to high-sensitive C-reactive-protein associated with coronary artery calcifications in healthy middle-aged subjects. *Atherosclerosis* 2014;237:60–6.
- Lyngbæk S, Marott JL, Sehestedt T, et al. Cardiovascular risk prediction in the general population with use of suPAR, CRP, and Framingham Risk Score. *Int J Cardiol* 2013;167:2904–11.
- Borné Y, Persson M, Melander O, et al. Increased plasma level of soluble urokinase plasminogen activator receptor is associated with incidence of heart failure but not atrial fibrillation. *Eur J Heart Fail* 2014;16:377–83.
- Glatz JF, van der Vusse GJ. Cellular fatty acid-binding proteins: their function and physiological significance. *Prog Lipid Res* 1996;35:243–82.
- Viswanathan K, Hall AS, Barth JH. An evidence-based approach to the assessment of heart-type Fatty Acid binding protein in acute coronary syndrome. *Clin Biochem Rev* 2012;33:3–11.
- Zschesche W, Kleine AH, Spitzer E, et al. Histochemical localization of heart-type fatty-acid binding protein in human and murine tissues. *Histochem Cell Biol* 1995;103:147–56.
- Antohe F, Popov D, Radulescu L, et al. Heart microvessels and aortic endothelial cells express the 15 kDa heart-type fatty acid-binding proteins. *Eur J Cell Biol* 1998;76:102–9.
- Bathia DP, Carless DR, Viswanathan K, et al. Serum 99th centile values for two heart-type fatty acid binding protein assays. *Ann Clin Biochem* 2009;46:464–7.
- Niizeki T, Takeishi Y, Takabatake N, et al. Circulating levels of heart-type fatty acid-binding protein in a general Japanese population: effects of age, gender, and physiologic characteristics. *Circ J* 2007;71:1452–7.
- Dieplinger B, Mueller T. Soluble ST2 in heart failure. *Clin Chim Acta* 2015;443:57–70.
- Lassus J, Gayat E, Mueller C, et al. Incremental value of biomarkers to clinical variables for mortality prediction in acutely decompensated heart failure: the Multinational Observational Cohort on Acute Heart Failure (MOCA) study. *Int J Cardiol* 2013;168:2186–94.
- Anand IS, Rector TS, Kuskowski M, et al. Prognostic value of soluble ST2 in the Valsartan Heart Failure Trial. *Circ Heart Fail* 2014;7:418–26.
- Kohli P, Bonaca MP, Kakkar R, et al. Role of ST2 in non-ST-elevation acute coronary syndrome in the MERLIN-TIMI 36 trial. *Clin Chem* 2012;58:257–66.
- Dieplinger B, Januzzi JL, Steinmair M, et al. Analytical and clinical evaluation of a novel high-sensitivity assay for measurement of soluble ST2 in human plasma—the Presage ST2 assay. *Clin Chim Acta* 2009;409:33–40.
- Sponder M, Campean IA, Emich M, et al. Long-term endurance training increases serum cathepsin S and decreases IL-6 and hsCRP levels. *J Sports Sci* 2017;35:2129–34.
- Sponder M, Campean IA, Emich M, et al. Long-term physical activity leads to a significant increase in serum sRAGE levels: a sign of decreased AGE-mediated inflammation due to physical activity? *Heart Vessels* 2018;33:893–900.
- Sponder M, Kopecky C, Campean IA, et al. Sports and HDL-Quality Reflected By Serum Amyloid A and Surfactant Protein B. *Int J Med Sci* 2017;14:1040–8.
- Sponder M, Campean IA, Dalos D, et al. Effect of long-term physical activity on PCSK9, high- and low-density lipoprotein cholesterol, and lipoprotein(a) levels: a prospective observational trial. *Pol Arch Intern Med* 2017;127:506–11.
- Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* 1989;5:303–11.
- Glatz JF, van der Vusse GJ. Intracellular transport of lipids. *Mol Cell Biochem* 1989;88:37–44.
- Binns B, Danneberg H, McWhir J, et al. Requirement for the heart-type fatty acid binding protein in cardiac fatty acid utilization. *Faseb J* 1999;13:805–12.
- Kleine AH, Glatz JF, Van Nieuwenhoven FA, et al. Release of heart fatty acid-binding protein into plasma after acute myocardial infarction in man. *Mol Cell Biochem* 1992;116:155–62.
- O'Donoghue M, de Lemos JA, Morrow DA, et al. Prognostic utility of heart-type fatty acid binding protein in patients with acute coronary syndromes. *Circulation* 2006;114:550–7.
- Górski J, Hermens WT, Borawski J, et al. Increased fatty acid-binding protein concentration in plasma of patients with chronic renal failure. *Clin Chem* 1997;43:193–5.

- 26 Sorichter S, Mair J, Koller A, *et al.* Early assessment of exercise induced skeletal muscle injury using plasma fatty acid binding protein. *Br J Sports Med* 1998;32:121–4.
- 27 Arı H, Tokaç M, Alihanoğlu Y, *et al.* Relationship between heart-type fatty acid-binding protein levels and coronary artery disease in exercise stress testing: an observational study. *Anadolu Kardiyol Derg* 2011;11:685–91.
- 28 Sbarouni E, Georgiadou P, Koutelou M, *et al.* Heart type fatty acid binding protein in relation to pharmacologic scintigraphy in coronary artery disease. *Clin Chem Lab Med* 2011;50:387–90.
- 29 Dungey M, Bishop NC, Young HM, *et al.* The impact of exercising during haemodialysis on blood pressure, markers of cardiac injury and systemic inflammation—preliminary results of a pilot study. *Kidney Blood Press Res* 2015;40:593–604.
- 30 Lippi G, Schena F, Montagnana M, *et al.* Influence of acute physical exercise on emerging muscular biomarkers. *Clin Chem Lab Med* 2008;46:1313–8.
- 31 Tominaga S. A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. *FEBS Lett* 1989;258:301–4.
- 32 Bartunek J, Delrue L, Van Durme F, *et al.* Nonmyocardial production of ST2 protein in human hypertrophy and failure is related to diastolic load. *J Am Coll Cardiol* 2008;52:2166–74.
- 33 Truong QA, Januzzi JL, Szymonifka J, *et al.* Coronary sinus biomarker sampling compared to peripheral venous blood for predicting outcomes in patients with severe heart failure undergoing cardiac resynchronization therapy: the BIOCRT study. *Heart Rhythm* 2014;11:2167–75.
- 34 Wang TJ, Wollert KC, Larson MG, *et al.* Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation* 2012;126:1596–604.
- 35 Coglianese EE, Larson MG, Vasan RS, *et al.* Distribution and clinical correlates of the interleukin receptor family member soluble ST2 in the framingham heart study. *Clin Chem* 2012;58:1673–81.
- 36 Chen LQ, de Lemos JA, Das SR, *et al.* Soluble ST2 is associated with all-cause and cardiovascular mortality in a population-based cohort: the dallas heart study. *Clin Chem* 2013;59:536–46.
- 37 Sanada S, Hakuno D, Higgins LJ, *et al.* IL-33 and ST2 comprise a critical biomechanically induced and cardioprotective signaling system. *J Clin Invest* 2007;117:1538–49.
- 38 Miller AM, Xu D, Asquith DL, *et al.* IL-33 reduces the development of atherosclerosis. *J Exp Med* 2008;205:339–46.
- 39 Liu Y, Liu SX, Cai Y, *et al.* Effects of combined aerobic and resistance training on the glycolipid metabolism and inflammation levels in type 2 diabetes mellitus. *J Phys Ther Sci* 2015;27:2365–71.