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ORIGINAL ARTICLE

Relationship between exercise capacity and urinary liver-type fatty acid-binding protein in middle-aged and older individuals

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Abstract

Background The underlying mechanism linking the decline in exercise capacity with renal dysfunction remains unclear. Urinary liver-type fatty acid-binding protein (L-FABP) levels reflect the degree of peritubular capillary blood flow, an important factor for renal dysfunction with aging. The aim of this study was to examine the relationship between exercise capacity and urinary L-FABP levels.

Methods This was a cross-sectional study of 187 middle-aged and older individuals (aged 50–83 years) without chronic kidney disease (CKD). We assessed urinary L-FABP levels, peak oxygen consumption ($\dot{V}O_{2peak}$), and grip strength.

Results Urinary L-FABP levels inversely correlated with both $\dot{V}O_{2peak}$ ($r_s = -0.349$) and grip strength ($r_s = -0.485$). When the participants were divided into four groups according to the median values of aerobic fitness and muscular strength ($\dot{V}O_{2peak}$ and grip strength), urinary L-FABP levels were the highest in participants with lower levels of aerobic fitness and muscular strength (2.95 ± 1.43 μg/g creatinine) and the lowest in the participants with higher levels of aerobic fitness and muscular strength (1.33 ± 0.76 μg/g creatinine). The difference between the two groups was significant ($P < 0.001$).

Conclusion Our results demonstrate that both $\dot{V}O_{2peak}$ and grip strength were inversely associated with urinary L-FABP levels in middle-aged and older individuals without CKD. This suggests that a decline in exercise capacity is associated with a reduction in peritubular capillary blood flow, providing a novel insight into the underlying mechanism linking the decline in exercise capacity to the development of renal dysfunction.

Keywords Aerobic fitness · Muscular strength · Peritubular capillary blood flow

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Introduction

A decline in exercise capacity, including aerobic fitness and muscular strength, has been associated with various poor health-related outcomes, including cardiovascular disease and all-cause mortality [1, 2]. Exercise capacity is also an independent predictor of the development and progression of chronic kidney disease (CKD) [3, 4]. Exercise capacity of patients with CKD is generally lower than that of healthy population norms or that of healthy control groups [5]. Thus, it is apparent that a decline in the exercise capacity can induce various renal outcomes; however, the pathophysiological mechanism that links the decline in exercise

capacity to the development of renal dysfunction remains unclear.

Age-related renal changes are initially characterized by a loss of peritubular capillaries due to intrarenal arterial changes, such as arteriolosclerosis and intimal and medial hypertrophy [6, 7]. A reduction in peritubular capillary blood flow leads to tubular hypoperfusion and hypoxia and eventually to tubular atrophy and tubulointerstitial fibrosis, which is the main cause of renal dysfunction induced with aging [8, 9]. A traditional viewpoint is that the progression of renal dysfunction is associated with the degree of tubulointerstitial, rather than glomerular damage [10]. Therefore, renal tubular evaluations may be more important than glomerular evaluations, such as estimated glomerular filtration rate (eGFR) and albuminuria, for identifying the development of renal dysfunction with aging.

Liver-type fatty acid-binding protein (L-FABP) is a 14-kDa protein expressed in the proximal tubular cells of the human kidney [11] and excreted into urine in response to tubular hypoxia caused by decreased peritubular capillary blood flow [12] and anemia [13]. Urinary L-FABP levels have shown a strong inverse correlation with peritubular capillary blood flow, directly measured by intravital video analysis in living-related kidney transplantation [12]. Furthermore, urinary L-FABP level is a highly sensitive renal tubular biomarker for predicting the progression of CKD [14–16]. Thus, as urinary L-FABP can reflect the degree of peritubular capillary blood flow, it could be a key biomarker for investigating the pathophysiological mechanism that links the decline in exercise capacity with the development of renal dysfunction with aging. However, a connection between exercise capacity and urinary L-FABP levels remains to be established.

Therefore, the aim of this study was to investigate a possible relationship between exercise capacity and urinary L-FABP levels in middle-aged and older individuals without CKD. For this purpose, we used a cross-sectional approach and assessed exercise capacity, including aerobic fitness and muscular strength, and urinary L-FABP levels.

Methods

Participants

A total of 187 middle-aged and older individuals without CKD participated in this study. All participants did not suffer from the renal disease, as assessed by their medical history. Participants who exceed the cut-off values for urinary L-FABP (≥ 8.4 μg/g creatinine), microalbuminuria (≥ 30 mg/g creatinine), or low values of $eGFR_{cys}$ (≤ 60 mL/min/1.73 m^2) were not included. This study was approved by the ethical committees of the Institute of Health and Sport Sciences of the University of Tsukuba. The study conformed to the principles outlined in the Helsinki Declaration, and all participants provided written informed consent before inclusion in the study.

Procedures

All measurements, excluding the incremental cycle exercise test, were performed in the morning after a 12-h overnight fast. Measurements were obtained in a quiet, temperature-controlled room (24–26 °C). At first, the participants collected their spot urine in individual. Subsequently, after a resting period of at least 20 min, brachial blood pressure and intrarenal resistive index were measured and blood samples were drawn to determine the blood chemistry. After these procedures, aerobic fitness and grip strength were measured using the cycling ergometer and grip dynamometer, respectively.

Clinical measurements

Body weight was measured to the nearest 0.1 kg using a digital scale. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as the participants' weight (in kg) divided by their height (in m^2). Visceral fat was measured using the dual-impedance analysis method (HSD-2000; Omron Healthcare, Kyoto, Japan) ($n = 186$). Waist circumference was directly measured on the skin at the level of the umbilicus with the participant in a standing position; measurements were made in duplicate to the nearest 0.1 cm. Brachial systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure, and heart rate (HR) were measured using a semi-automatic vascular testing device equipped with an electrocardiogram and oscillometric extremities cuffs (Form PWV/ABI; Colin Medical Technology, Aichi, Japan).

Blood samples were also drawn to measure total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride, fasting blood glucose ($n = 186$), blood urea nitrogen ($n = 105$), creatinine, and cystatin C levels. To exclude the influence of muscle mass, eGFR was calculated using the new equation based on standardized serum cystatin C levels as follows [17]: $eGFR_{cys}(mL/min/1.73\ m^2) = [104 \times \text{serum cystatin C}^{-1.019} \times 0.996^{Age} (\times 0.929, \text{if female})] - 8$. Urinary excretion of albumin ($n = 178$), β2-microglobulin ($n = 105$), N-acetyl-β-D-glucosaminidase (NAG) ($n = 103$), and creatinine levels were also measured using spot urine samples.

Urinary L-FABP assays

Urinary L-FABP levels were measured using a highly sensitive sandwich enzyme-linked immunosorbent assay (High Sensitivity Human L-FABP ELISA Kit; CMIC Company, Limited, Tokyo, Japan). Diluted standards and samples were incubated in an antibody-immobilized plate at room temperature (25 °C) for 1 h. They were then washed three times with phosphate-based buffer containing the detergent and allowed to react with horseradish peroxidase-conjugated monoclonal antibody for 30 min. After washing three times, it was reacted with 3,3′,5,5′-tetramethylbenzidine solution at room temperature (25 °C) for 30 min, and the reaction terminated by adding sulfuric acid. Absorbance was measured at 450 nm on a microplate reader. The limit of detection was 0.3 ng/mL. Urinary L-FABP levels were evaluated as the ratio of urinary L-FABP (μg) to urinary creatinine levels (g).

Intrarenal resistive index

In a subset of participants ($n = 101$), intrarenal artery (i.e., segmental artery) duplex ultrasound was performed to examine the relationship between exercise capacity and intrarenal resistive index. Using duplex ultrasonography with a 3.5-MHz convex array probe (Noblus C25, Hitachi Aloka Medical, Tokyo, Japan), intrarenal peak systolic flow velocities and end-diastolic flow velocities were recorded in at least three different segmental renal arteries in each kidney, as previously reported [18]. The intrarenal resistive index was calculated for both kidneys from the equation: Intrarenal resistive index = 1− (intrarenal end-diastolic flow velocities/intrarenal peak systolic flow velocities) [19]. The mean values were used in the subsequent analysis.

Aerobic fitness and muscular strength

To evaluate their aerobic fitness, the participants performed an incremental cycling ergometer exercise consisting of 2 min at 20 W followed by a 10-W increase every 1 min; we measured peak oxygen consumption ($\dot{V}O_{2peak}$) in METs using online computer-assisted circuit spirometry (AE300S; Minato Medical Science, Osaka, Japan). MET is defined as the energy expended at rest, which is approximately equivalent to the oxygen consumption of 3.5 mL of O_2 per kg body weight per minute [3]. The following criteria were applied for the termination of the exercise test: (1) the participant reaching their age-predicted maximal HR (i.e., 220−age), (2) Borg's scale > 19, (3) respiratory equivalent > 1.2, or (4) the participant being unable to maintain a pedalling speed > 55 rpm [20]. As an indicator of muscular strength, we measured grip strength using a Takei dynamometer (T.K.K.5401; Takei Kiki Kogyo, Niigata, Japan). This was assessed with reference to the guideline of the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

Statistical analysis

Data are presented as the means ± SD (for normal distributions), median with interquartile range (for skewed distributions), or frequency counts (for categorical data). The participants were stratified according to whether their $\dot{V}O_{2peak}$ and grip strength values were above or below the median for each of these variables. Univariate analyses were performed using Spearman's rank correlation coefficients (r_s) and Kruskal–Wallis nonparametric tests with post hoc paired comparisons as appropriate. Partial correlation analysis was used to test the association between exercise capacity and urinary L-FABP levels, adjusting for the confounding effects of potentially relevant variables. Statistical significance was set a priori at $P < 0.05$ for all comparisons. Statistical analyses were performed using SPSS software (version 21).

Results

The participants' clinical measurements are summarized in Table 1. The mean age was 62 ± 7 years (range, 50–83 years). Mean blood biochemistry results were within the normal range, including HDL cholesterol (65 mg/dL), LDL cholesterol (135 mg/dL), triglycerides (96 mg/dL), and fasting blood glucose (93 mg/dL), and mean brachial SBP/DBP was 124/76 mmHg. Renal parameters were also within the normal range (Table 2): $eGFR_{cys}$ (99 mL/min/1.73 m^2), urinary albumin (7.5 mg/g creatinine), and urinary L-FABP (2.1 μg/g creatinine). A few of the participants were taking medication, such as antihypertensive (3%) or antidyslipidemic medicines (3%); and 2% of the participants were smokers.

Figure 1 shows the univariate correlations between exercise capacity and urinary L-FABP levels (A, B), urinary albumin levels (C, D), and $eGFR_{cys}$ (E, F). Urinary L-FABP levels were inversely correlated with both $\dot{V}O_{2peak}$ ($r_s = -0.349$, $P < 0.001$) and grip strength ($r_s = -0.492$, $P < 0.001$). Even after adjusting for age, BMI, mean arterial pressure and total cholesterol, triglyceride, fasting blood glucose, and serum creatinine levels, there were significant correlations between the urinary L-FABP levels and exercise capacity ($\dot{V}O_{2peak}$: partial $r_s = -0.275$, $P < 0.001$ and grip strength: partial $r_s = -0.213$, $P = 0.004$). Furthermore, the similar results were obtained on replacement of serum creatinine levels with urinary creatinine levels (data are not shown). Urinary albumin levels were not correlated with $\dot{V}O_{2peak}$ ($r_s = -0.093$, $P = 0.218$) or grip strength

Table 1 Clinical measurements of participants

Variable	Total
n	187
Women, *n* (%)	103 (55)
Age, year	62 ± 7
Height, cm	161 ± 8
Weight, kg	60 ± 9
Body mass index, kg/m^2	23 ± 3
Visceral fat, cm^2 [a]	57 ± 25
Waist circumference, cm	83 [78–89]
Total cholesterol, mg/dL	221 ± 33
High-density lipoprotein cholesterol, mg/dL	65 ± 14
Low-density lipoprotein cholesterol, mg/dL	135 ± 31
Triglyceride, mg/dL	86 [61–118]
Fasting blood glucose, mg/dL[a]	92 [86–99]
Systolic blood pressure, mmHg	124 ± 15
Diastolic blood pressure, mmHg	76 ± 9
Mean arterial pressure, mmHg	92 ± 10
Heart rate, bpm	60 [54–64]
$\dot{V}O_{2peak}$, MET	6.66 [5.94–7.66]
Grip strength, kg	30.4 [25.1–36.7]
Smoking, *n* (%)	5 (3)
Antihypertensive medicine, *n* (%)	4 (2)
Antidyslipidemic medicine, *n* (%)	3 (2)

Data are shown as the mean ± SD, median [interquartile range], or frequency counts (%), as appropriate

[a]Data available in 186 individuals

Table 2 Renal parameters of participants

Variable	Total
Serum creatinine, mg/L	0.70 ± 0.15
Serum cystatin C, mg/L	0.75 [0.67–0.81]
$eGFR_{cys}$, mL/min/1.73 m^2	99 ± 16
Blood urea nitrogen, mg/dL[c]	15.1 ± 3.6
Urinary L-FABP, μg/g creatinine	1.67 [1.08–2.72]
Urinary albumin, mg/g creatinine[b]	6.03 [4.09–8.49]
Urinary *β*2-microglobulin, mg/g creatinine[c]	111 [81–164]
Urinary NAG, U/g creatinine[d]	4.76 [3.24–6.67]
Intrarenal peak systolic flow velocities, cm/s[e]	36.6 [33.8–39.2]
Intrarenal end-diastolic flow velocities, cm/s[e]	15.2 ± 2.4
Intrarenal resistive index[e]	0.58 [0.55–0.62]

Data are shown as the mean ± SD or median [interquartile range], as appropriate

eGFR$_{cys}$ estimated filtration rate calculated by cystatin C, *L-FABP* liver-type fatty acid-binding protein, *NAG* N-acetyl-*β*-D-glucosaminidase

[b]Data available in 178 individuals

[c]Data available in 105 individuals

[d]Data available in 103 individuals

[e]Data available in 101 individuals

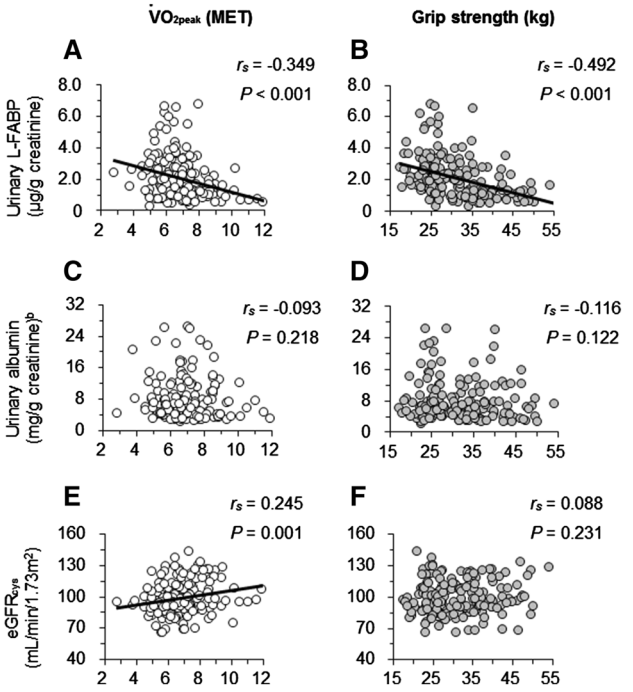

Fig. 1 Relationships between exercise capacity and urinary liver-type fatty acid-binding protein (L-FABP) levels (**a, b**), urinary albumin levels (**c, d**), and estimated filtration rate calculated by cystatin C ($eGFR_{cys}$) (**e, f**). [b]Data available in 178 individuals

($r_s = -0.116$, $P = 0.122$). $eGFR_{cys}$ was positively correlated with $\dot{V}O_{2peak}$ ($r_s = 0.245$, $P = 0.001$), but not with grip strength ($r_s = 0.088$, $P = 0.231$). The other urinary renal tubular parameters, β2-microglobulin and NAG, were not significantly correlated with exercise capacity: urinary β2-microglobulin levels ($\dot{V}O_{2peak}$: $r_s = -0.044$, $P = 0.656$ and grip strength: $r_s = 0.013$, $P = 0.899$) and urinary NAG levels ($\dot{V}O_{2peak}$: $r_s = -0.061$, $P = 0.541$ and grip strength: $r_s = 0.046$, $P = 0.644$).

The participants' intrarenal flow measurements are summarized in Table 2. The mean values of intrarenal peak systolic flow velocities, end-diastolic flow velocities, and the resistive index were 36.9 ± 4.4 cm/s, 15.2 ± 2.4 cm/s, and 0.59 ± 0.05, respectively. When the participants were divided into two groups according to the median values of aerobic fitness and muscular strength ($\dot{V}O_{2peak}$ and grip strength), the mean value of the intrarenal resistive index was significantly lower in the higher aerobic fitness and higher muscular strength group than in the lower aerobic fitness and lower muscular strength group (0.60 ± 0.06 vs. 0.57 ± 0.04, $P = 0.016$; 0.60 ± 0.05 vs. 0.57 ± 0.05, $P = 0.001$, respectively).

The participants were divided into four groups (the group with lower levels of both aerobic fitness and muscular strength, with lower aerobic fitness and higher muscular strength, with higher aerobic fitness and lower

muscular strength, and with higher levels of both aerobic fitness and muscular strength) according to whether their aerobic fitness and muscular strength values ($\dot{V}O_{2peak}$ and grip strength) were above or below the median values for these variables (6.7 MET and 30.4 kg, respectively). The mean age was 62 ± 7, 64 ± 7, 61 ± 6, and 62 ± 8 years. The mean BMI was 24 ± 3, 24 ± 3, 22 ± 3, and 23 ± 2 kg/m^2. Mean blood biochemistry results were as follows: HDL cholesterol (62 ± 14, 62 ± 13, 72 ± 15, and 64 ± 14 mg/dL), LDL cholesterol (144 ± 28, 128 ± 37, 134 ± 32, and 130 ± 27 mg/dL), triglycerides (95 ± 45, 106 ± 62, 82 ± 37, and 98 ± 46 mg/dL), and fasting blood glucose (91 ± 10, 96 ± 11, 90 ± 10, and 96 ± 11 mg/dL). Mean brachial blood pressure were as follows: SBP (124 ± 16, 125 ± 15, 123 ± 16, and 124 ± 12 mmHg), DBP (73 ± 9, 78 ± 9, 75 ± 10, and 79 ± 8 mmHg), and MAP (90 ± 11, 94 ± 10, 91 ± 11, and 94 ± 9 mmHg).

The combined influence of aerobic fitness and muscular strength on urinary L-FABP levels (A), intrarenal resistive index (B), urinary albumin levels (C), and $eGFR_{cys}$ (D) is shown in Fig. 2. Urinary L-FABP levels, intrarenal resistive index, and $eGFR_{cys}$ differed significantly between the four groups ($P < 0.001$, $P = 0.002$, and $P = 0.029$, respectively), but urinary albumin levels did not. The urinary L-FABP levels was the highest (2.95 ± 1.43 μg/g creatinine) in the group with lower levels of both aerobic fitness and muscular strength and the lowest (1.33 ± 0.76 μg/g creatinine) in the group with higher levels of both aerobic fitness and muscular strength; this difference was statistically significant ($P < 0.001$). There was no significant difference in urinary L-FABP levels between the group with higher aerobic fitness and lower muscular strength (2.15 ± 1.20 μg/g creatinine) and the group with lower aerobic fitness and higher muscular strength (2.01 ± 1.30 μg/g creatinine), but the urinary L-FABP levels in both these groups were significantly lower than those in the group with lower levels of both aerobic fitness and muscular strength ($P = 0.046$ and $P = 0.001$, respectively). The intrarenal resistive index was the highest (0.61 ± 0.06) in the group with lower levels of both aerobic fitness and muscular strength and the lowest (0.56 ± 0.04) in the group with higher levels of both aerobic fitness and muscular strength; this difference was statistically significant ($P = 0.001$).

Discussion

The underlying mechanism that links the decline in exercise capacity with renal dysfunction remains unclear. Renal aging is initially characterized by the loss of peritubular capillaries [6, 7], and therefore, in this study, we focused on urinary L-FABP levels, which reflects the degree of peritubular capillary blood flow [12] and we examined its relationship with exercise capacity. There were two key findings. First, urinary L-FABP levels were inversely correlated with both $\dot{V}O_{2peak}$ and grip strength. Second, when

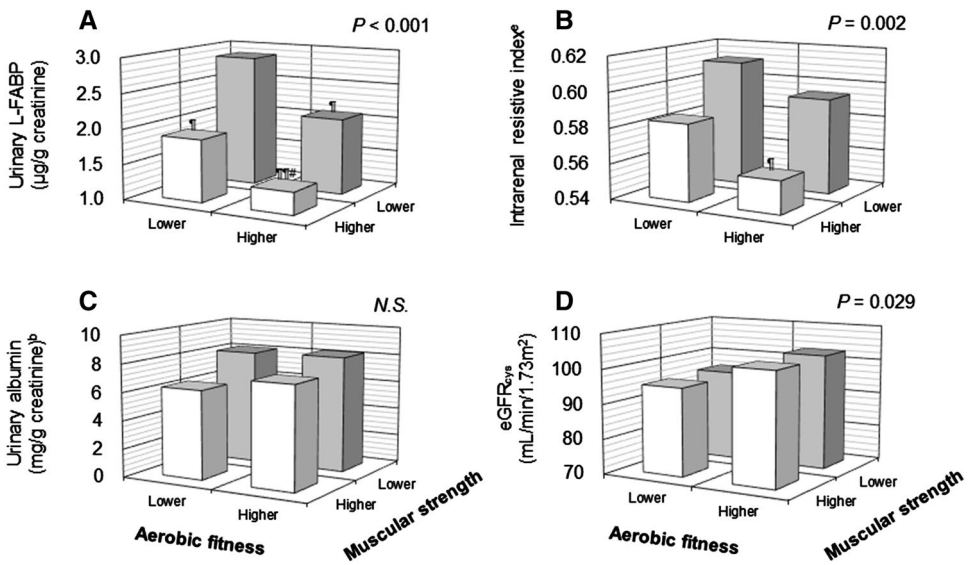

Fig. 2 Urinary liver-type fatty acid-binding protein (L-FABP) levels (**a**), intrarenal resistive index (**b**), urinary albumin levels (**c**), and estimated filtration rate calculated by cystatin C ($eGFR_{cys}$) (**d**) for the four groups of participants. The participants were divided into groups according to whether their levels of aerobic fitness and muscular strength (as measured by $\dot{V}O_{2peak}$ and grip strength) was greater or less than the median values for these variables. *P* values were evaluated by Kruskal–Wallis nonparametric tests for four-group comparisons. $^{¶¶}P < 0.001$, $^{¶}P < 0.05$ vs. the group with lower levels of both aerobic fitness and muscular strength. $^{\#}P < 0.01$ vs. the group with higher levels of aerobic fitness and lower levels of muscular strength. [b]Data available in 178 individuals, [e]Data available in 101 individuals

the participants were divided into four groups according to the median values of $\dot{V}O_{2peak}$ and grip strength (aerobic fitness and muscular strength), the urinary L-FABP levels were the highest in the group with lower levels of both aerobic fitness and muscular strength. These results suggest that a decline in exercise capacity is associated with a reduction in peritubular capillary blood flow in middle-aged and older individuals without CKD.

It may not be possible to identify a direct relationship between exercise capacity and urinary L-FABP in patients with CKD, because a number of factors increase urinary L-FABP levels, such as proteinuria/albuminuria [21], hyperglycemia [22], and anemia [13], in addition to peritubular capillary blood flow [12]. We, therefore, recruited participants without CKD to investigate this relationship. Even when both eGFR and urinary albumin levels were within the normal range, exercise capacity was inversely associated with urinary L-FABP levels. Furthermore, in a subset of participants ($n=101$), we observed that an intrarenal resistive index, the renovascular resistance parameter [23], which is related to peritubular capillary loss [24], was also inversely associated with exercise capacity. Although the exercise capacity may not be associated with glomerulus function, because the kidney has a functional reserve in the glomerulus [25], our results suggest that it is inversely associated with the degree of peritubular capillary blood flow in middle-aged and older individuals without CKD. Thus, the reduction in peritubular capillary blood flow could be a partial explanation for the pathophysiological mechanism that links the decline in exercise capacity with the development of renal dysfunction.

Here, we demonstrated that urinary L-FABP levels and the intrarenal resistive index were the lowest in the participants with higher levels of aerobic fitness and muscular strength. These results suggest that there is an additive effect of aerobic fitness and muscular strength in maintaining sufficient peritubular capillary blood flow. The previous study has also shown that the prevalence of metabolic syndrome, an independent risk factor for renal dysfunction, was the lowest in individuals with higher levels of aerobic fitness and muscular strength [26]. These results suggest that not only aerobic exercise training but also resistance exercise training is important for retarding renal dysfunction with aging.

Higher aerobic fitness and muscular strength in middle-aged and older individuals may result in more muscle mass, thereby increasing serum creatinine levels and urinary creatinine excretion and reducing urinary L-FABP levels on adjusted urinary creatinine excretion may be induced. However, the association between exercise capacity and urinary L-FABP levels was significant even after adjusting for the confounding effects of serum and urinary creatinine levels and other potentially relevant variables. Furthermore, other urinary parameters on adjusted urinary creatinine excretion, such as urinary albumin, β2-microglobulin, and NAG levels did not correlate significantly with exercise capacity. Therefore, it is conceivable that the relationship between exercise capacity and urinary L-FABP levels is not able to be explained only by the differences in serum and urinary creatinine levels.

Although we were unable to identify the precise etiology of the association between exercise capacity and urinary L-FABP levels, we propose some possible theories. First, urinary L-FABP levels are affected by oxidative stress [27, 28], and our group recently observed that urinary L-FABP levels were positively correlated with urinary thiobarbituric acid reactive substance levels, known to be an oxidative stress marker ($n=45$; $r_s = 0.363$, $P=0.014$) (K. Kosaki, unpublished data). Conversely, oxidative stress levels are increased by a decline in exercise capacity [29]. Oxidative stress may, therefore, be involved in the association between exercise capacity and urinary L-FABP levels. Second, endothelium-derived nitric oxide (NO), a vasodilator related to peritubular capillary blood flow, may contribute to the present observation. Our previous study reported that exercise training that increased exercise capacity also led to an increase in plasma nitrite/nitrate levels (NOx: the stable end-product of NO) [30, 31]. This increase in endothelium-derived NO arising from a high level of exercise capacity could result in lower urinary L-FABP levels (i.e., sufficient peritubular capillary blood flow).

However, this study includes several limitations. First, this was a single-centre study that included a relatively small sample size. Second, the specific etiology of the relationship between exercise capacity and urinary L-FABP levels, such as oxidative stress and endothelium-derived NO, was not investigated. Third, we had adjusted the present observations by the serum and urinary creatinine levels, because the sex difference of urinary L-FABP levels can be explained by the urinary creatinine levels [32]. Thereby, the relationship between exercise capacity and urinary L-FABP levels remained significant after the consideration of serum and urinary creatinine levels. However, we cannot completely deny the influence of gender on the present observations. Fourth, in this study, we used the intrarenal resistive index at segmental renal artery as a surrogate parameter of peritubular capillary flow, because the previous study has reported that intrarenal resistive index at segmental renal artery is related to the degree of both arteriosclerosis and peritubular capillary loss in patients with CKD [24]. However, it is unclear whether intrarenal resistive index affects peritubular capillary flow even in non-CKD subjects. Finally, because of the cross-sectional nature of this investigation, no clear conclusions could be reached regarding any causal links between changes in exercise capacity and urinary L-FABP levels. Accordingly,

further multi-centre and prospective studies with larger sample sizes are needed in each sex to identify the precise etiology of the association between exercise capacity and urinary L-FABP levels.

In conclusion, exercise capacity, as measured by $\dot{V}O_{2peak}$ and grip strength, was inversely associated with urinary L-FABP levels in middle-aged and older individuals without CKD. These results suggest that a decline in exercise capacity in middle-aged and older individuals without CKD is associated with a reduction in peritubular capillary blood flow. We believe that this study provides an important insight into the underlying mechanism that links the decline in exercise capacity to the development of renal dysfunction.

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Compliance with ethical standards

Conflict of interest Takeshi Sugaya is the director and senior scientist of CMIC Company Limited (Tokyo, Japan), the company that produced the high Sensitivity Human L-FABP ELISA Kit for LFABP analysis. The authors have declared that no conflict of interest exists.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee at which the studies were conducted (IRB approval number Tai25-127, Tai26-122, Tai27-68) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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