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Research paper

Oncostatin M mediates cardioprotection via angiogenesis in ischemic heart disease

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ABSTRACT

Objective: Oncostatin M (OSM) is an inflammatory cytokine belonging to the interleukin-6 family member, which plays an important role in various cardiovascular diseases. We recently reported increased serum OSM levels in patients with coronary artery disease. However, the specific role in HF with ischemic heart disease (IHD) remains unclear.

Methods and results: A total of 120 patients with HF and 48 control subjects were enrolled in this study. Serum OSM levels were measured using a sandwich technique immunoassay during the compensated state. The results revealed significantly higher serum OSM levels in HF patients compared to controls. Importantly, HF patients with IHD had higher OSM levels, and those with collateral flow showed the even higher levels, indicating a potential involvement in angiogenesis. Furthermore, a positive correlation was found between serum OSM levels and levels of vascular endothelial growth factor (VEGF). In vitro experiments demonstrated that recombinant OSM upregulated VEGF production in cultured human coronary artery endothelial cells. We additionally observed that endogenous OSM levels were enhanced through exercise. Lastly, we identified the potential of SGLT2 inhibitors to enhance OSM production.

Conclusions: Serum OSM levels were elevated in HF patients, particularly in those with IHD Our data indicated that endogenous OSM induces VEGF production in the heart, suggesting the activation of angiogenesis, which can be further enhanced by exercise or SGLT2 inhibitors.

1. Introduction

Heart failure (HF) is a multifaceted clinical syndrome that arises from various cardiovascular diseases and is becoming increasingly prevalent in developed nations due to the aging population, posing a significant mortality risk [1].

Oncostatin M (OSM), a member of the interleukin-6 family of cytokines, plays a pivotal role in diverse biological processes, including cell growth and inflammatory responses related to cardiovascular diseases [2]. A prior study has demonstrated elevated plasma OSM levels in HF patients with reduced ejection fraction (HFrEF) [3]. Additionally, our previous researches demonstrated the crucial involvement of the OSM-Yes-associated protein (YAP), a key transcriptional co-factor in the Hippo pathway, in the development of cardiac dysfunction through cardiac dedifferentiation in a murine model [4,5]. Moreover, we recently reported increased serum OSM levels in patients with coronary artery diseases (CAD) [6]. However, the role of OSM in ischemic heart disease (IHD) related HF remains uncertain. Therefore, we aimed to investigate it here.

2. Methods

2.1. Study population

This study enrolled consecutive patients with HF who were admitted to the International University of Health and Welfare Hospital between April 2019 and Jul 2022. All patients had recently been hospitalized for HF and were treated with intravenous diuretics to confirm the presence of HF. Patients with acute coronary syndrome, recent revascularization, constrictive pericarditis, myocarditis, end-stage renal disease requiring dialysis, malignant tumors, preoperative patients, or those with inflammatory diseases such as collagen disease were excluded. Subjects without HF who underwent coronary angiography were included as a

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Abbreviations

ACEI	angiotensin-converting enzyme inhibitor
ARB	angiotensin receptor blocker
ARNI	angiotensin receptor neprilysin inhibitor
BB	β-blocker
BNP	brain natriuretic peptide
CAD	coronary artery disease
CCB	calcium channel blocker
DM	diabetes mellitus
EF	ejection fraction
HCAEC	human coronary artery endothelial cells
HF	heart failure
hs-CRP	high-sensitive C-reactive protein
IHD	ischemic heart disease
LVDd	left ventricular end-diastolic diameter
LVEF	left ventricular ejection fraction
OSM	oncostatin M
YAP	yes-associated protein
VEGF	vascular endothelial growth factor

comparison group. A total of 120 HF patients (77 men, 43 women; mean age, 75.6 ± 5.0 years) and 48 control subjects (30 men, 18 women; mean age, 75.4 ± 5.1 years) were enrolled in this study. All HF patients underwent elective coronary angiography (CAG) at the International University of Health and Welfare Hospital in Japan. IHD was defined by narrowing of the coronary lumen by >75 % or by performing fractional flow reserve (FFR)/ instantaneous wave-free ratio (iFR). The etiology of HF was as follows: 70 patients had ischemic heart disease (IHD), 20 had valvular heart disease (mitral regurgitation in 10, aortic regurgitation in 4, and aortic stenosis in 6), and 30 had cardiomyopathy (hypertensive heart disease in 18, hypertrophic cardiomyopathy in 2, and dilated cardiomyopathy in 10). Physical activity was assessed according to the American College of Cardiology/American Heart Association guidelines for the diagnosis and management of chronic HF in adults, and all patients were classified as stage C. Control subjects were matched with HF patients in terms of age, gender, body mass index (BMI), and cardiac risk factors such as hypertension, diabetes mellitus (DM), dyslipidemia, and smoking. DM was diagnosed based on the Japanese guidelines for diabetes, which considered a previous diagnosis or treatment with hypoglycemic drugs or insulin use. In this study, a BMI of ≥25 was considered obese.

2.2. Study approval

This study, including the procedure for enrollment, conformed to the principles of the Declaration of Helsinki and was approved by the Ethics Committees of the International University of Health and Welfare Hospital. All patients provided written informed consent. The authors had full access to the data and take full responsibility for its integrity.

2.3. Measurement of serum levels of OSM and VEGF

In this study, all patients underwent coronary angiography for diagnosis after receiving intensive care and cardiac rehabilitation. Blood samples were obtained in the morning after an overnight fast, while the patients were in a stable condition, essentially before their discharge. The blood samples were then centrifuged for 10 min at 2500*g* within 30 min of collection. Serum samples were stored at −80 °C. The levels of OSM and VEGF in the serum were measured using the human OSM ELISA kit (Lot: ab215543, Abcam, USA) and VEGF ELISA kit (Lot: ab100662, Abcam, USA), respectively, following the manufacturer's instructions.

2.4. Cell culture

Human coronary artery endothelial cells (HCAEC) were purchased from Takara Biochemicals (C-12221 Lot:479Z0261). The HCAEC were cultured at 37 °C using Takara medium (C-22121). The cultured HCAEC were treated with 10 ng/ml of recombinant OSM (Cell Signaling Technology #5367) for 24 h. Culture supernatant was collected and centrifuged at 1500*g* for 5 min at 4 °C. The levels of VEGF in the culture supernatant were also using the VEGF ELISA kit (Lot: ab100662, Abcam, USA).

2.5. Exercise

In this study, all HF patients engaged in cardiac rehabilitation among hospitalization. Patients took light-moderate intensity physical activity load for 30 min, such as leisure-brisk walking exercise (2–3 mph, about 3Mets). For the measurement of OSM level, the blood sample was obtained before and after exercise from 48 patients, who were capable of exercising under continuous intensity loads.

2.6. Data collection

The medical records were used to collect baseline demographic data including age, gender, BMI, medication (angiotensin-converting enzyme inhibitor [ACEI], angiotensin receptor blocker [ARB], angiotensin receptor neprilysin inhibitor [ARNI], calcium channel blocker [CCB], β-blocker [BB], and statin). Left ventricular ejection fraction (LVEF) was measured on echocardiography. According to the guidelines, we divided the patients into two groups: HF with preserved ejection fraction (HFpEF, LVEF ≥50 %), HF with mid-range ejection fraction (HFmrEF, 50 % > LVEF ≥40 %) and HF with reduced ejection fraction (HFrEF, 40 % > LVEF).

2.7. Statistical analysis

Results are expressed as mean ± standard error of the mean. Continuous variables are presented as median and interquartile range, and categorical variables are presented as number and percentage. Baseline characteristics were compared among quartiles using the chi square test for categorical variables and the Wilcoxon or Kruskal–Wallis rank-sum test for continuous variables. Student's *t*-test was used for comparisons between two groups, and Dunnett's multiple comparison of means was used for multi-group comparison after analysis of variance. Statistical analysis was performed using SPSS (SPSS, Chicago, IL, USA). A p value of <0.05 was considered to be statistically significant.

3. Results

3.1. Clinical characteristics

Table 1 presents the baseline clinical characteristics. The average age of all patients was 75.5 ± 5.0 years, with 64 % being men. The study included 120 patients with HF and 48 control subjects. Among the HF patients, the average LVEF was 53.0 ± 12.5 %. A total of 28 HF patients had atrial fibrillation. Furthermore, 90 patients were diagnosed with DM (diabetes mellitus). Among these patients, the average HbA1c level was 6.8 ± 1.1 %, and the mean duration of diabetes was 5.7 ± 2.6 years.

3.2. The serum OSM level was elevated in HF

The serum OSM level in HF patients during the chronic stable phase was significantly higher compared to the control subjects. (Fig. 1A) Furthermore, we categorized the patients into HFrEF, HFmrEF, and HFpEF groups to examine the serum OSM levels within each group. Notably, HF patients with HFrEF exhibited significantly higher serum OSM levels. (Supplementary Fig. 1) Next, we compared the serum levels

Table 1
Characteristics of patients in the present study.

	Control	HF	Total	*P*-value
Number	48	120	168	
Male (%)	30 (63)	77 (64)	107 (64)	0.840
Age, years	75.4 ± 5.1	75.6 ± 5.0	75.5 ± 5.0	0.813
BMI, kg/m^2	23.6 ± 2.5	24.3 ± 2.9	24.1 ± 2.8	0.223
NYHA functional class				
I		30 (25)	30 (18)	
II		73 (61)	73 (43)	
III		15 (13)	15 (13)	
IV		2 (2)	2 (1)	
Medical history				
Hypertension (%)	42 (88)	107 (89)	130 (89)	0.760
Diabetes mellitus (%)	23 (48)	67 (56)	90 (54)	0.356
Dyslipidemia (%)	26 (54)	67 (56)	93 (55)	0.846
Smoking (%)	27 (56)	64 (53)	91 (54)	0.734
Medical history				
Myocardial infarction (%)	0	44 (37)	44 (26)	<0.001
Atrial fibrillation (%)	1 (2)	28 (23)	29 (17)	<0.001
Hemodynamics and LV function				
SBP, mmHg	123.3 ± 10.5	124.5 ± 9.4	124.1 ± 9.7	0.476
HR, bpm	72.2 ± 7.3	72.7 ± 7.9	72.5 ± 7.7	0.724
LVDd, mm	43.9 ± 2.2	54.4 ± 7.9	50.8 ± 8.1	<0.001
LVEF, %	71.6 ± 5.7	53.0 ± 12.5	58.9 ± 13.8	<0.001
Laboratory finding				
Hb, g/dl	13.1 ± 1.0	13.2 ± 0.8	13.2 ± 0.9	0.497
Creatinine, mg/dl	0.93 ± 0.12	1.02 ± 0.20	0.99 ± 0.15	0.478
BNP, pg/ml	9.3 ± 3.5	164.3 ± 89.6	120.0 ± 102.6	<0.001
HbA1c, %	6.49 ± 0.9	6.94 ± 1.1	6.80 ± 1.1	0.017
hs-CRP, mg/dl	0.11 ± 0.2	0.11 ± 0.2	0.11 ± 0.2	0.240
OSM, pg/ml	94.6 ± 27.5	138.0 ± 57.1	124.9 ± 53.9	<0.001
Treatment				
ACEI/ARB/ARNI (%)	34 (71)	98 (82)	132 (79)	0.124
β-blocker (%)	15 (31)	94 (78)	109 (65)	<0.001
CCB (%)	24 (50)	61 (51)	85 (51)	0.923
Statin (%)	23 (48)	59 (49)	82 (49)	0.884
MRA (%)	5 (10)	62 (52)	67 (40)	<0.001
SGLT2 inhibitor (%)	5 (10)	34 (28)	39 (23)	0.013

of OSM between HF patients with IHD and HF patients without IHD. Importantly, the serum OSM level was significantly higher in HF patients with IHD compared to those without IHD. (Fig. 1B) Specifically, among HF patients with IHD, the serum OSM level demonstrated a negative correlation with LVEF. (Supplementary Fig. 2).

3.3. Serum OSM level and VEGF level were high in HF with IHD

Notably, we observed that HF patients with collateral flow had particularly elevated serum OSM levels, indicating a potential role of angiogenesis. (Supplementary Fig. 3) Consequently, our focus shifted to vascular endothelial growth factor (VEGF), a key mediator of angiogenesis, and we assessed its levels in HF patients. The serum VEGF level was also significantly higher in HF patients with IHD than in those without IHD. (Fig. 2A) We subsequently identified a positive correlation between serum OSM levels and VEGF levels. (Fig. 2B).

3.4. Recombinant OSM upregulated VEGF production in vitro study

In order to investigate the capacity of OSM to induce VEGF in the heart, we conducted an in vitro study. Specifically, we treated human coronary artery endothelial cells (HCAEC) with recombinant OSM (10 ng/ml for 24 h) and observed an upregulation of VEGF in the culture supernatant. (Fig. 3).

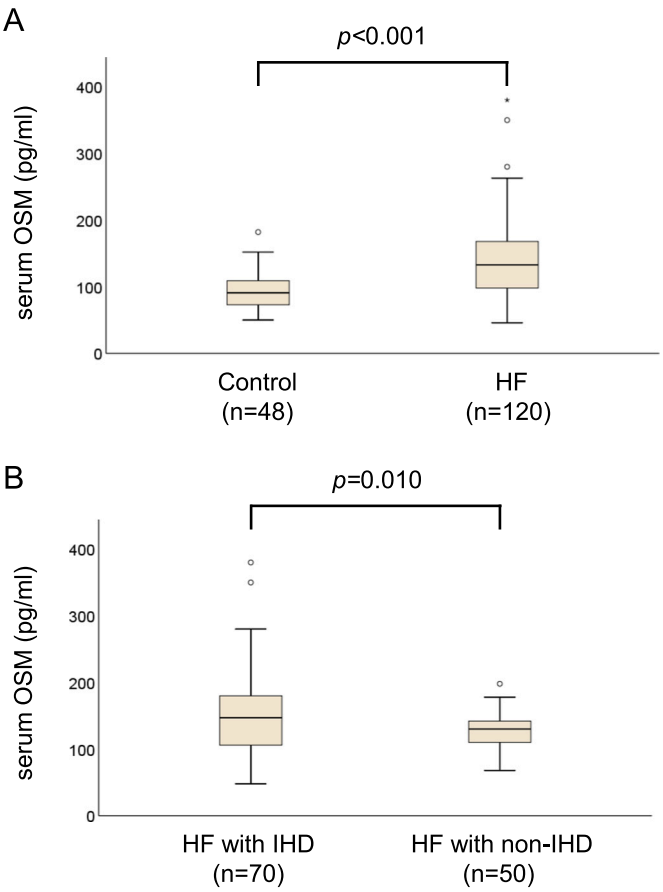

Fig. 1. (**A**) Serum OSM levels were higher in HF patients than in control subjects. (**B**) Serum OSM levels were higher in HF patients with IHD than in HF patients with non-IHD.

3.5. Exercise increased serum OSM and VEGF levels in HF patients

Recent reports indicate that aerobic exercise has the potential to elevate OSM levels in the skeletal muscle of mice [7]. Consequently, we proceeded to measure the post-exercise serum levels of OSM in 48 HF patients and compared the results among them. Supplementary Table presents the baseline characteristics of 48 HF patients. Our data showed that exercise resulted in a notable increase in serum OSM levels among HF patients. (Fig. 4A) Furthermore, we observed that the increase in OSM levels induced by exercise was greater in HF patients with IHD compared to those without IHD. (Fig. 4B).

SGLT2 inhibitor had a potential role to increase serum OSM level in HF patients.

Nowadays, it is widely known that Sodium-glucose cotransporter 2 (SGLT2) inhibitors, originally developed as a treatment for DM, have been shown to reduce mortality and hospitalization rates in patients with HF [8,9]. However, the precise mechanisms underlying these effects have not been fully elucidated. Therefore, we here hypothesized that SGLT2 inhibitors may have an impact on serum OSM levels in individuals with HF. In this study, we investigated the change of serum OSM level by the additional use of SGLT2 inhibitors in HF patients. Subsequently, we conducted a study to investigate the effects of administering SGLT2 inhibitors (empagliflozin 10 mg or dapagliflozin 10 mg) to 40 patients with HF over an additional 3-month period.

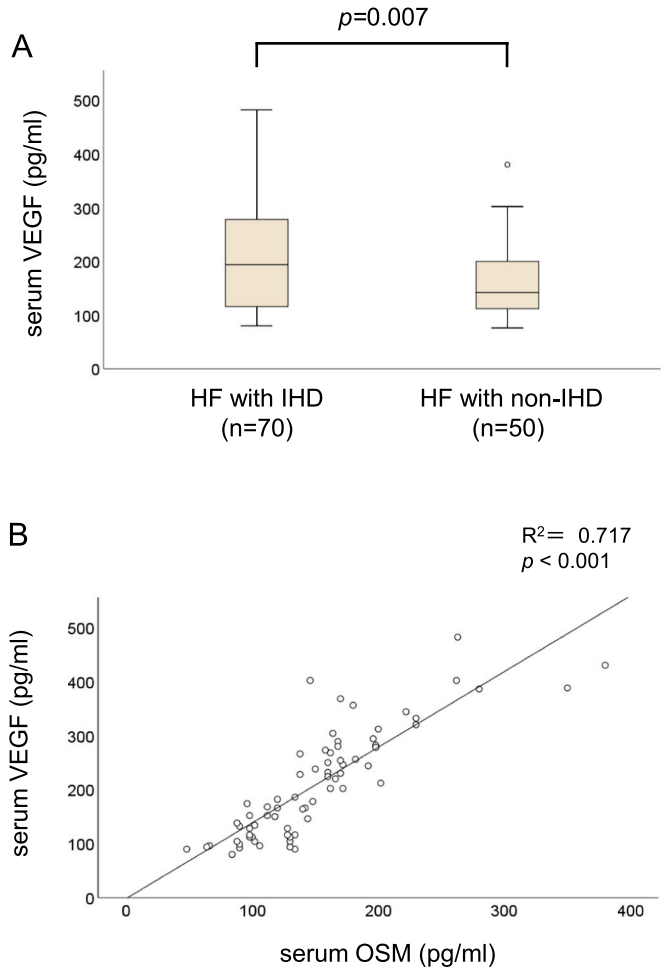

Fig. 2. (**A**) Serum VEGF levels were higher in HF patients with IHD than in those with non-IHD. (**B**) There was a positive correlation between serum OSM and VEGF among HF patients.

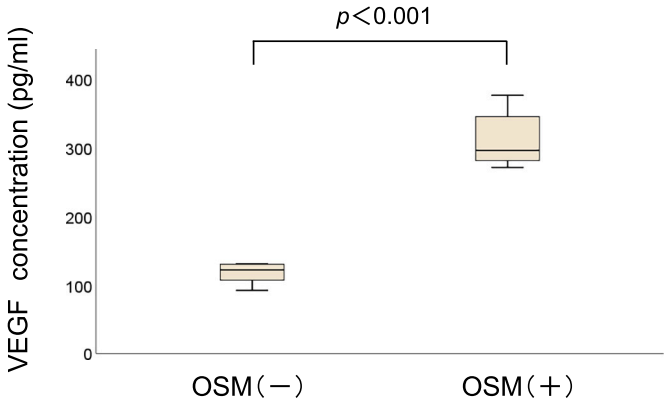

Fig. 3. Recombinant OSM (10 ng/ml for 24 h) upregulated VEGF concentration in the culture supernatant. Experiments were repeated 3 times.

Importantly, the administration of SGLT2 inhibitors for an additional 3 months in patients with HF could potentially elevate serum OSM levels, suggesting a potential role in stimulating endogenous OSM production (Fig. 5).

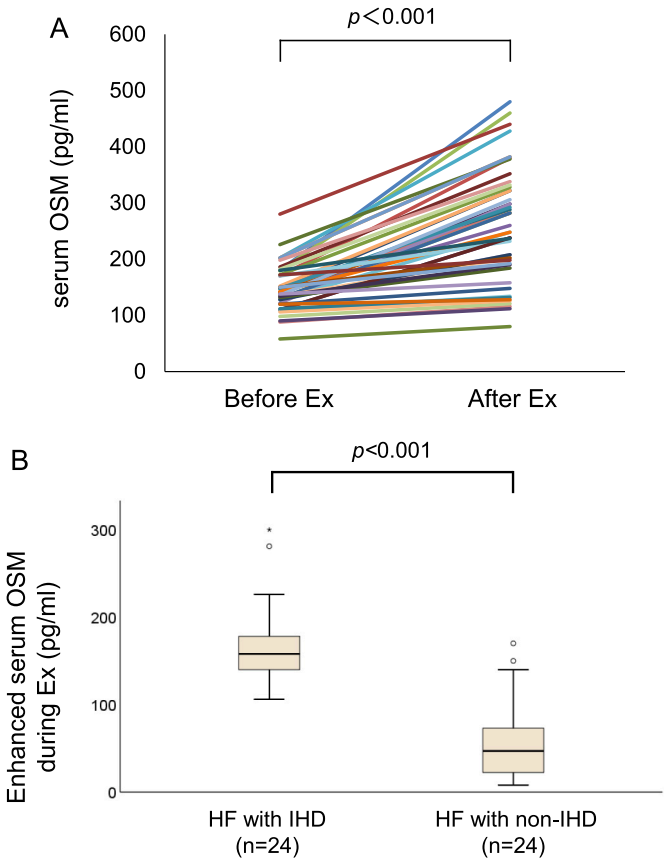

Fig. 4. (**A**) Exercise increased serum OSM and VEGF levels in HF patients (n = 48). (**B**) The enhancement of serum OSM levels during exercise was higher in HF patients with IHD than in those with non-IHD (n = 24 each).

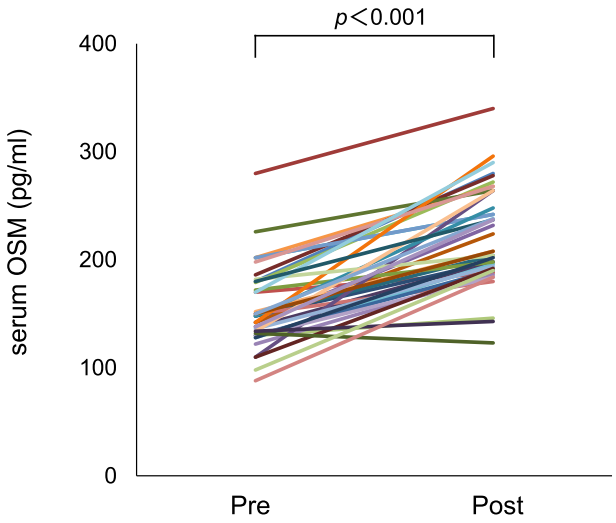

Fig. 5. The change in serum OSM levels in HF patients before and after additional SGLT2 inhibitors (3 months).

4. Discussion

The major findings of this study are as follows: 1) serum OSM levels were significantly elevated in HF patients, particularly in those with HFrEF, 2) both serum OSM and VEGF levels were elevated in HF patients with IHD, 3) recombinant OSM upregulated VEGF production in HCAEC, 4) exercise led to an increase in serum OSM and VEGF levels

among HF patients, and 5) SGLT2 inhibitors had the potential to elevate serum OSM levels in HF patients.

A previous report has demonstrated elevated plasma OSM levels in patients with HFrEF [3]. Consistent with this finding, we have also found significantly elevated serum OSM levels in HF patients, particularly in those with HFrEF. Furthermore, we observed higher serum OSM levels in HF patients with IHD compared to those with non-IHD. These results align with our previous study where we reported an increase in serum OSM levels in patients with CAD [6]. Therefore, these findings support our previous research and do not contradict it. Additionally, Kubin et al. previously demonstrated that OSM activation was caused in HF related to myocardial infarction in vivo, which further reinforces the consistency of our results [10]. Notably, we observed significantly elevated serum OSM levels in HF patients with collateral flow, suggesting a potential involvement of angiogenesis. A prior study reported that OSM treatment in human astroglioma cell lines increased VEGF levels [11]. In this study, we investigated the association of OSM and VEGF, and indeed, we discovered a positive correlation between the levels of serum OSM and VEGF. Moreover, we identified recombinant OSM upregulated VEGF production in HCAEC. Thus, we assume that OSM may play a cardioprotective role by facilitating VEGF-induced angiogenesis. Then, we contemplated methods to enhance endogenous OSM as a potential treatment for HF.

A wealth of studies indicates that engaging in exercise leads to many beneficial effects on the heart [12]. Recent studies demonstrate that certain myokines induced by exercise play an important role in combating several diseases, including cardiovascular diseases [13]. It has reported that aerobic exercise can increase the levels of OSM in the skeletal muscle of mice [7]. Therefore, in this study, we investigated the effect of exercise on serum OSM levels in HF patients. Importantly, our data showed that exercise enhanced serum OSM levels in all HF patients, suggesting an increase in endogenous OSM as an exercise-induced myokine. Consistent with our findings, OSM has been reported to stimulate angiogenesis by upregulating VEGF and bFGF, suggesting its potential as a therapeutic strategy for treating ischemic diseases in vivo [14]. Based on these findings, we thought that this enhanced endogenous OSM, induced by exercise, could lead VEGF-induced angiogenesis, especially in HF patients.

Nowadays, it is widely known that the use of SGLT2 inhibitors reduces cardiovascular events and hospitalizations due to HF [8,9]. However, the underlying mechanism of the drug's effect on HF remains unknown. A recent study has demonstrated that SGLT2 inhibitors facilitate angiogenesis by activating VEGF in kidney [15]. Therefore, we conducted an investigation to explore the hypothesis that SGLT2 inhibitors may upregulate the YAP-OSM pathway in patients with HF. Our findings revealed that extending the administration of SGLT2 inhibitors for an additional 3 months in HF patients has the potential to increase serum OSM levels. This also suggests a potential role by stimulating endogenous OSM production and promoting angiogenesis in patients with HF.

4.1. Study limitations

Several limitations in the present study should be mentioned. First, in this small study, we examined comprehensive data obtained from a single institution. Further larger-scale studies are needed to validate our findings. Second, while we assumed that OSM may play a cardioprotective role by facilitating VEGF-induced angiogenesis, the details have not been fully clarified. Third, the source of OSM remains unknown, although we propose skeletal muscle as one potential source. Fourth, the control of OSM by factors other than YAP is still unclear. Therefore, caution should be exercised when equating serum OSM levels with the activity of the YAP-OSM pathway, and further clarification is needed. Fifth, it is unknown whether the long-term exercise has a similar effect or not. Finally, although our study suggests that angiogenesis through the OSM-VEGF pathway is one of the potential mechanisms for the effects of SGLT2 inhibitors on heart failure, the overall understanding of the effects of SGLT2 inhibitors on heart failure is still incomplete. These points should be addressed in future studies as well.

4.2. Conclusion

Serum OSM levels were elevated in HF patients, particularly those with IHD. Endogenous OSM may exert a cardioprotective effect by facilitating angiogenesis, a process that can be further stimulated through exercise. Additionally, SGLT2 inhibitors have the potential to augment the production of endogenous OSM.

CRediT authorship contribution statement

Shohei Ikeda: Conceptualization, Methodology, Investigation, Writing – original draft, Funding acquisition. **Koichi Sato:** Investigation. **Morihiko Takeda:** Investigation. **Mariko Shinozaki:** Investigation. **Keita Miki:** Investigation. **Michinori Hirano:** Investigation. **Koji Fukuda:** Investigation. **Nobuyuki Shiba:** Investigation.

Declaration of competing interest

The authors declare that there are no conflicts of interest associated with this study.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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IRB information

This study was approved by the ethics committee in International University of Health and Welfare Hospital and assigned as approval number: 13B351.

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Ethical statement

The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ahjo.2023.100331.

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