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Effect of combined aerobic and resistance exercise on serum Klotho secretion in healthy young men -a pilot study-

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ABSTRACT

The impact of combined aerobic and resistance exercise on Klotho (KL) secretion is unclear. Twelve healthy young men completed two randomized experimental trials: 1) resistance exercise (RE) and 2) resistance exercise with prior aerobic exercise (AE + RE). Following baseline blood pressure assessment and blood collection, the subjects in the RE trial maintained a supine position for 45 min, while the subjects in the AE + RE trial performed 45 min of aerobic exercise. After 45 min of resting or aerobic exercise, all subjects performed resistance exercise. Following resistance exercise, the subjects rested in a supine position for 60 min. Blood pressure assessment and blood collection were repeated. Aerobic and resistance exercise significantly increased serum KL concentrations, respectively ($P < 0.05$), and no additive effect of aerobic exercise on KL secretion was observed immediately after resistance exercise in the AE + RE trial compared with the RE trial. However, serum KL levels at 30 and 60 min after resistance exercise were significantly higher in the AE + RE trial than in the RE trial. Serum ET-1 concentrations were significantly increased only in the RE trial. In conclusion, combined aerobic and resistance exercise could maintain higher levels of serum KL secretion after exercise compared with resistance exercise only.

1. Introduction

Klotho (KL) is an anti-aging protein that is primarily produced by the kidney (Kurosu et al., 2005; Kuro, 2019). Klotho plays an important role in energy (glucose and fat) metabolism (Razzaque, 2012). Additionally, higher blood KL concentrations are associated with lower cardiometabolic risk in sedentary individuals (Amaro-Gahete et al., 2020). Indeed, higher levels of blood Klotho concentration is closely related to high endothelial function, which means blood Klotho level is an early predictor of atherosclerosis (Keles et al., 2015). Exercise is well known as an important "therapy" in the treatment of cardiometabolic diseases associated with aging. However, the relationship between exercise and KL secretion is unknown. Matsubara et al. (2014) reported that long-term aerobic training augments plasma KL concentrations in postmenopausal women. In addition, serum KL concentrations are increased following moderate-intensity training, but not sprint interval training (Middelbeek et al., 2021). In studies that focused on the impact of acute exercise, a significant increase in serum KL concentrations is observed in men and women (Santos-Dias et al., 2017; Tan et al., 2018). By contrast, a single bout of exhaustive exercise in mice causes a significant reduction in serum KL levels (Rao et al., 2019). We recently demonstrated that acute high-intensity resistance exercise increases serum KL concentrations in healthy young men (Morishima and Ochi, 2021). On the other hand, a previous study reported a significant reduction of plasma KL concentrations immediately after acute jump exercise (Iturriaga et al., 2021). Although these studies were conducted with the understanding of the influence of aerobic and resistance exercise on the KL response, an important limitation is that no prior study examined the impact of combined aerobic and resistance exercise on blood KL secretion. It is of paramount importance to focus on the KL response following combined exercise because this is a very common training style for many people.

Therefore, we performed a preliminary examination of the impact of a single bout of combined aerobic and resistance exercise on serum KL levels in the present study. We also measured the levels of serum endothelin-1 (ET-1), which is a powerful vasoconstrictor (Yanagisawa et al., 1988). A single bout of resistance exercise increases plasma ET-1 concentration (Boeno et al., 2019; Okamoto et al., 2008; Morishima

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et al., 2021), and the augmentation of ET-1 levels inhibit Klotho expression (Wang and Sun, 2014). However, ET-1 response to combined aerobic and resistance exercise is still unclear. We hypothesized that serum KL levels would be higher after combined aerobic and resistance exercise than after resistance exercise only and ET-1 could be associated with this response.

2. Materials and methods

2.1. Subjects

We recruited 12 young, healthy males (20.4 ± 0.3 years, 174.6 ± 2.3 cm, 64.1 ± 2.5 kg, 17.5 ± 2.4 kg/m^2) in this study. All subjects were explained regarding the experimental procedure and the purpose of this study, and their written informed consent was subsequently obtained. All study procedures were approved by the Ethics Committee for Human Experiments at the Sports Research Center at Hosei University in Japan [ID: 2017-003]. The exclusion criteria in this study were as follows: (a) individuals that were participating regular exercise training programs, (b) smoker, (c) patients (or history) with cardiovascular, pulmonary, metabolic, or neurological disease, (d) individuals that were taking medications and supplements.

2.2. Experimental procedures

The present study was consisted of three visits. The one repetition maximum (1RM) of leg extension was assessed in the first visit. Following the warm-up sets, the workload was increased until the subjects were unable to perform a lift.

At least 7days after the first visit, the experimental trials (second and third visits) were performed (the order was randomized). The study procedure was shown in Fig. 1. In the second and third visits, subjects performed the resistance exercise trial (RE trial) or the resistance exercise after the aerobic exercise trial (AE + RE trial). Subjects were allowed to have a light meal 2 or more h before the beginning of these trials, which has been used in many previous studies (Restaino et al., 2015, 2016; Morishima et al., 2018, 2019a, 2019b). All subjects did not take any caffeine or alcohol drinks at least 10 h. In addition, all subjects refrained exercise for 24 h. We conducted the second and third visits at same timing in a day. The temperature at the laboratory was kept at 23 °C. At first, the subjects were maintained in a supine position for 10 min. Then we measured systolic and diastolic blood pressure (SBP and DBP) using an automated sphygmomanometer (Omron Cooperation, Kyoto, Japan) and obtained baseline blood sample from antecubital vein using polyethylene catheter.

After the baseline measurement, subjects maintained the supine position for 45 min in the RE trial, while in the AE + RE trial, subjects performed aerobic exercise (cycling) for 45 min (Aerobike 75XLIII, Combi wellness Co, Tokyo, Japan). A cycling cadence was kept at 60 rpm. Subjects were asked to performed this aerobic exercise at a rate of perceived exertion (RPE) of 11–13 (Borg Scale, 6–20). We recorded the heart rate (HR) and RPE during aerobic exercise. Following the 45 min of supine resting or aerobic exercise, the subjects conducted resistance exercise (leg extensions). The timing at the biggening of this resistance exercise was matched between trials (Fig. 1). The protocol of resistance exercise was 10 repetitions for 5 sets at 70% of 1RM. Resting periods among all sets were 60 s. We measured SBP and DBP during the intervals. Subjects got back to the supine position after the resistance exercise. Then we repeated blood pressure measurements and blood collections at 0, 30, and 60 min after resistance exercise.

2.3. Data analysis

The %HRmax during aerobic exercise was calculated from the age-predicted HRmax ([the average HR during aerobic exercise/(220 - age) × 100]). Serum samples were obtained by centrifugation for 10 min and stored at −80 °C until analysis. Serum KL (#2799; Immuno-Biological Laboratories, Takasaki, Japan) and ET-1 (DET100; R&D, Minneapolis, MN, USA) concentrations were measured by enzyme-linked immunosorbent assays. The intra-assay coefficients of variation were 3.2% and 3.8% for serum KL and ET-1, respectively. We calculated the relative (i.e., percentage) change of serum KL and ET-1 concentrations to investigate the effect of combined aerobic and resistance exercise. In the RE trial, the relative change from Baseline 1 to RE-0 min was calculated (i.e., the effect of resistance exercise only). In the AE + RE trial, the relative changes from Baseline 1 to AE-0 min (i.e., the effect of aerobic exercise only) and from Baseline 1 to RE-0 min (i.e., the effect of combined aerobic and resistance exercise) were calculated.

2.4. Statistical analysis

A two-way (time × trial) analysis of variance (ANOVA) with repeated measures was performed to analyze all dependent data. When a significant interaction or main effect was detected, we performed Tukey's post hoc test. The level of significance was accepted at $P \leq 0.05$. Data are shown as means ± standard deviation.

3. Results

The average workload during aerobic exercise was 83.7 ± 3.0 W.

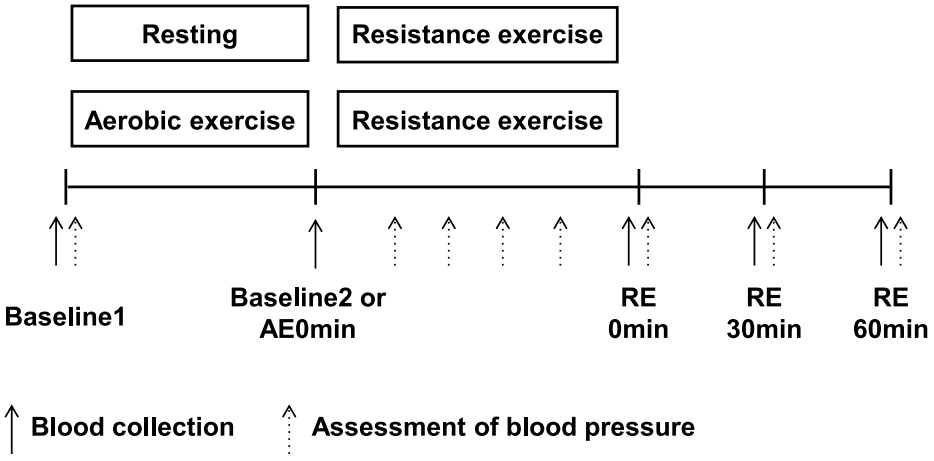

Fig. 1. Experimental design
The intensity of resistance exercise was five sets of 10 repetitions at 70% of 1RM. The workload of cycling was individualized for each subject to target a rate of perceived exertion of 11–13.

Absolute and relative exercise intensity was 119.3 ± 5.0 bpm and 67.0 ± 1.7% HRmax, respectively. The average rate of perceived exertion during aerobic exercise was 12.8 ± 0.2. The average workload during resistance exercise was 69.7 ± 4.0 kg.

Systolic blood pressure was significantly elevated in response to resistance exercise in both trials ($P < 0.05$), but returned to baseline levels thereafter (Table 1). Although there was a significant elevation in diastolic blood pressure in both trials immediately after the first set of resistance exercise ($P < 0.05$), there were no significant changes at the other time points (Table 1).

In the AE + RE trial, aerobic exercise significantly increased serum KL concentrations, and subsequent resistance exercise resulted in a further elevation ($P < 0.05$). Although KL levels (i.e., RE-0 min in the AE + RE trial) did not differ at the same time point in the RE trial, serum KL concentrations at 30 and 60 min after resistance exercise were significantly higher in the AE + RE trial than in the RE trial (Fig. 2A). The relative changes (%) of serum KL levels were higher after RE and AE + RE than after AE, but significant differences were not detected (Fig. 2B).

Serum ET-1 levels did not change following prior aerobic exercise and subsequent resistance exercise in the AE + RE trial ($P > 0.05$). However, a significant increase in serum ET-1 concentrations was observed in response to resistance exercise in the RE trial ($P < 0.05$, Fig. 3A). Similarly, only RE induced a significant increase in the relative change (%) of serum ET-1 levels ($P < 0.05$, Fig. 3B).

4. Discussion

The main findings of the present study are twofold. Firstly, the elevation of serum KL levels immediately after combined aerobic and resistance exercise was not significantly different from the increase of serum KL levels following resistance exercise only. However, secondly, serum KL concentrations at 30 and 60 min after resistance exercise were significantly higher in the AE + RE trial than in the RE trial. These data include a significant physiological finding that adding aerobic exercise before resistance exercise could maintain higher levels of circulating KL in the later phase.

Increasing attention has been paid to the effects of KL on the prevention of cardiometabolic diseases. In this line, previous studies have investigated the impact of aerobic and/or resistance exercise on blood KL responses (Matsubara et al., 2014; Santos-Dias et al., 2017; Baghaiee et al., 2018; Tan et al., 2018; Ramez et al., 2019; Rao et al., 2019). However, no study has examined the impact of combined resistance and aerobic exercise on blood KL concentrations. Therefore, we investigated serum KL levels following combined resistance and aerobic exercise. In the AE + RE trial, aerobic exercise significantly increased serum KL concentrations. Therefore, we consider that exercise caused the increase of KL regardless of exercise mode or combination.

Interestingly, serum KL concentrations were significantly higher in the AE + RE trial than in the RE trial at 30 and 60 min after resistance exercise. Previous studies have shown that either aerobic or resistance exercise causes an increase in KL levels (Matsubara et al., 2014; Santos-Dias et al., 2017; Tan et al., 2018; Morishima and Ochi, 2021). The detailed mechanism is unclear, but we suggest that adding aerobic exercise before resistance exercise may have a role in the elevation of circulating KL levels. In the further studies, only AE trial should be added to examine this point. In addition, future studies need to examine if these findings can be applicable to other population because this study included only healthy young males.

We observed that ET-1 concentrations did not change in the AE + RE trial, but were significantly increased in the RE trial. ET-1 is a strong vasoconstrictor, and a single bout of resistance exercise increases plasma ET-1 concentrations (Okamoto et al., 2008; Boeno et al., 2019), and the augmentation of ET-1 levels inhibits KL expression (Wang and Sun, 2014). Consistent with these studies, RE resulted in a significant elevation of ET-1 concentrations. By contrast, low-to-moderate intensity aerobic exercise has been shown to increase NO synthesis, which

Table 1
Systolic and diastolic Blood pressure before, during and after resistance exercise.

			Duration of resistance exercise																ANOVA
		Baseline1	1st set	2nd set	3rd set	4th set	RE0min	RE30min	RE60min	
Systolic Blood Pressure (mmHg)	RE trial	120 ± 8	143 ± 13*	148 ± 13*	144 ± 13*	150 ± 10*	136 ± 15	120 ± 6	120 ± 7	Time: $P < 0.01$
	AE + RE trial	124 ± 5	144 ± 19*	143 ± 12*	146 ± 16*	137 ± 20*	136 ± 16	118 ± 7	119 ± 7	Trial: N.S.
										Interaction: N.S.
Diastolic Blood Pressure (mmHg)	RE trial	70 ± 7	80 ± 6*	74 ± 9	74 ± 10	76 ± 18	65 ± 9	69 ± 8	72 ± 6	Time: $P < 0.01$
	AE + RE trial	71 ± 7	84 ± 7*	78 ± 8	79 ± 11	73 ± 8	71 ± 4	67 ± 9	70 ± 7	Trial: N.S.
										Interaction: N.S.

Mean ± SD. *$P < 0.05$ vs. Baseline1.

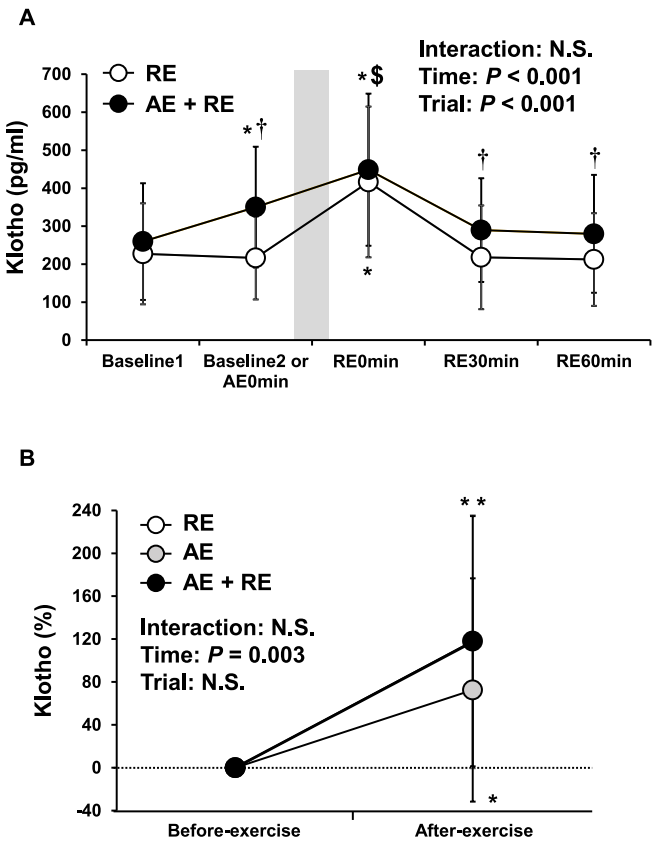

Fig. 2. (A) Serum klotho concentrations in each trial. (B) Relative (%) change of serum klotho concentrations before and after each exercise.
RE indicates the percentage change from Baseline 1 to RE-0 min in the RE trial. AE indicates the percentage change from Baseline 1 to AE-0 min in the AE + RE trial. AE + RE indicates the percentage change from Baseline 1 to RE-0 min in the AE + RE trial.
Data are expressed as means ± standard deviation. Two-way (time × trial) repeated measures analysis of variance with Tukey's post hoc test was performed. *P < 0.05 vs. Baseline 1. †P < 0.05 vs. RE trial. $P < 0.05 vs. AE-0 min. Shaded box indicates the duration of resistance exercise.

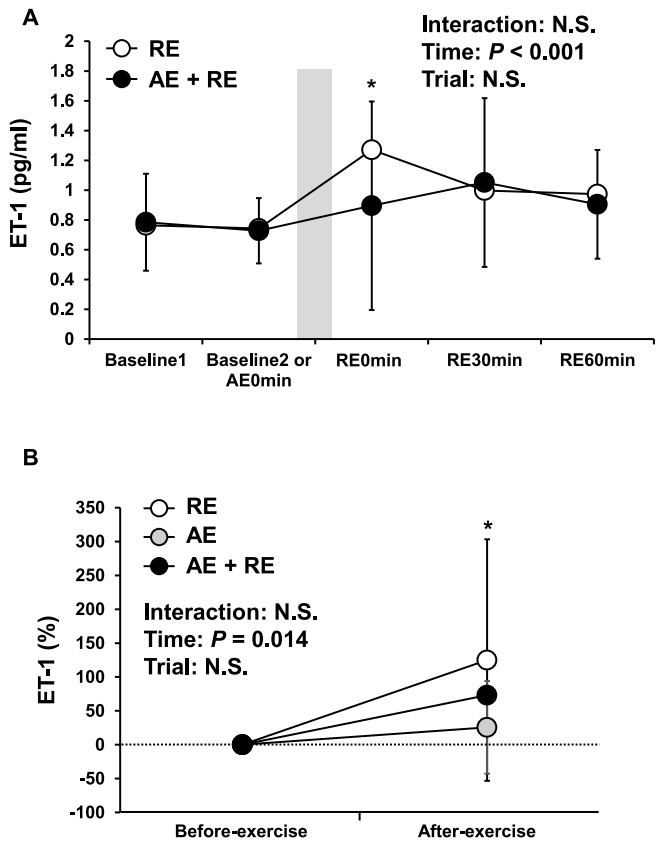

Fig. 3. (A) Serum ET-1 concentrations in each trial. (B) Relative (%) change of serum ET-1 concentrations before and after each exercise.
RE indicates the percentage change from Baseline 1 to RE-0 min in the RE trial. AE indicates the percentage change from Baseline 1 to AE-0 min in the AE + RE trial. AE + RE indicates the percentage change from Baseline 1 to RE-0 min in the AE + RE trial.
Data are expressed as means ± standard deviation. Two-way (time × trial) repeated measures analysis of variance with Tukey's post hoc test was performed. *P < 0.05 vs. Baseline 1. Shaded box indicates the duration of resistance exercise.

induces vasodilation (Giles et al., 2018), and ET-1 levels are not affected or decreased after a single bout of aerobic exercise (Ballard et al., 2017). To the best of our knowledge, this is the first study to investigate the ET-1 response to resistance exercise with prior aerobic exercise. From the results of the present study, we think that the effects of vasodilation from prior aerobic exercise would alleviate the ET-1 response after subsequent resistance exercise.

In conclusion, the present study revealed that serum KL concentrations were significantly increased following a single bout of combined aerobic and resistance exercise. However, the magnitude of the increase in serum KL levels did not differ significantly from the serum KL concentrations immediately after resistance exercise only. However, combined aerobic and resistance exercise maintained higher levels of serum KL secretion at 30 and 60 min after exercise compared with resistance exercise only.

Ethics statement

All procedures performed in this study were in accordance with the ethical standards of the committee of Hosei University, Japan (ID: 2017-003).

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CRediT authorship contribution statement

Takuma Morishima: Formal analysis, Investigation, Data curation, Software, Visualization, Writing – original draft. **Eisuke Ochi:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition, Investigation, Resources, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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