Effects of hypervolemia by protein and glucose supplementation during aerobic training on thermal and arterial pressure regulations in hypertensive older men

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ABSTRACT
The incidence of heat illness in older people has rapidly increased during midsummer for the last decade in Japan, and we suggested that whey-protein + carbohydrate supplementation during aerobic training increased plasma volume (PV) to enhance thermoregulatory adaptation in older men (J Appl Physiol, 107: 725-733, 2009); however, >60% of people age 65 and older suffer from hypertension and the symptoms may be worsened by the hypervolemia. To examine this, we randomly divided 21 older men (~69 years) with ~160 mmHg for systolic and ~90 mmHg for diastolic blood pressure at rest into two groups; Glc (N=11) consuming glucose alone (25g) and Pro-Glc (N=10) consuming whey-protein (10g) + glucose (15g), immediately after cycling exercise at 60-75% of peak aerobic capacity (\(\dot{V}O_2\)peak) for 60 min·day\(^{-1}\), 3 days·week\(^{-1}\), for 8 weeks. Before and after training, we measured PV (dye dilution), baroreflex sensitivity (BRS) of heart rate (Valsalva maneuver), and carotid arterial compliance (CAC) from carotid arterial diameter (ultrasound imaging) responses to pulsatile arterial pressure change (photoplethysmography) at rest. Additionally, we measured esophageal temperature (Tes) and forearm skin blood flow (plethysmography) during exercise at 60% pre-training \(\dot{V}O_2\)peak for 20 min in a warm environment. We found that the forearm skin vascular conductance response to increased Tes was enhanced in Pro-Glc with increased PV, but this was not found in Glc; however, despite the increased PV, arterial blood pressures rather decreased with increased CAC and BRS in Pro-Glc. Thus, the prescription was applicable to older men with hypertension to prevent heat illness during exercise.

Key words: aerobic training, hypertension, older men, supplement, thermoregulation
New & Noteworthy

The incidence of heat illness has rapidly increased in older people during the midsummer. Protein+carbohydrate supplementation during aerobic training reportedly increased plasma volume to enhance thermoregulatory adaptation; however, in hypertensive older people, the symptoms may be worsened by the hypervolemia. We demonstrated that in hypertensive older men, protein+glucose supplementation during aerobic training increased plasma volume and thermoregulation but blood pressures rather decreased. Thus, the prescription was applicable to hypertensive older people to prevent heat illness.
INTRODUCTION

The incidence of heat illness has rapidly increased to ~50,000 per year during the midsummer for the last decade in Japan as the atmospheric temperature ($T_a$) increased. Notably, ~50% of the incidence occurred in people age 65 and older (27, 33) due to reduced cardiovascular and body temperature regulations under heat stress with aging (11, 38). In addition, hypertension, which affects more than 60% of people age 65 and older (17, 30), reportedly accelerates the loss of heat dissipation mechanisms with aging (22). However, there are few countermeasures to prevent this by improving thermoregulatory capacity in older people with hypertension.

We reported that, in ~68-year-old normotensive men, a mixture of whey-protein + carbohydrate intake immediately after moderate intensity of exercise (30 min·day$^{-1}$, 3 days·week$^{-1}$, 8 weeks) increased plasma volume (PV) by ~6% to improve cutaneous vasodilatory and sweating responses to increased esophageal temperature ($T_{es}$) by 80% and 18%, respectively, but placebo treatment had no such effect, suggesting that the prescription was one of the countermeasures against heat illness for older people (37); however, clinicians have expressed their concerns that the increased PV could worsen the symptoms of hypertension in older people by increasing cardiac output and causing renal adaptations (14).

On the other hand, it has been suggested that endurance training at moderate intensity decreases arterial blood pressure in hypertensive and normotensive middle-aged and older people (15, 43), likely because it decreases sympathetic nervous out flow (25) and increases arterial compliance (46) and baroreflex sensitivity (29). These results suggest that endurance exercise training would improve arterial blood pressure regulation mechanisms sufficiently enough to prevent a possible increase in arterial pressure by the increase in PV by the
prescription in hypertensive older people.

Based on these, we hypothesized that the prescription of a mixture of whey-protein and glucose intake during exercise training increases PV and improves thermoregulatory responses, but does not increase arterial blood pressures with increased arterial compliance and/or baroreflex sensitivity in older men with mild to moderate hypertension. If we obtain the results to support the hypothesis, the prescription of the mixture intake during aerobic training will be applicable to a large population of older people to prevent heat illness during the midsummer.

METHODS

Subjects and Protocol:

We recruited subjects from older people who had participated in the health promotion program for elderly people in Matsumoto named ‘Jukunentaiikudaigaku’ for more than 6 months (34) before the recruitment. In the program, they were encouraged to perform interval walking training with a target of repeating 5 sets of fast and slow walking for 3 min each at > 70% and ~40% VO$_{2peak}$, respectively, per day, more than 4 days·week$^{-1}$. We recruited the subjects who suffered from hypertension, with blood pressure readings that nearly matched the category of hypertension specified by the American Heart Association (AHA) (6); not lower than 140 mmHg for systolic blood pressure (SBP) and/or 90 mmHg for diastolic blood pressure (DBP) at the measurement in April or September in the program prior to the recruitment. The procedure in this study was approved by the Institutional Review Board on Human Experiments, Shinshu University School of Medicine, and conformed to the standards set by the Declaration of Helsinki in 1989. After the experimental protocol was fully explained, 26 of 61 responders to the recruitment gave
written, informed consent before participating in the study. By interviewing with them regarding their past and current health status using questionnaires, we confirmed that all subjects were nonsmokers and had no overt history of diseases that would limit the exercise tests and training in the present study.

The experiments were conducted from the end of September 2011 to the beginning of July 2014. Fig. 1 shows a timeline of the measurements for 5 days before and after exercise training. For the pretraining assessment, on the first day, the subjects were instructed to come to the laboratory at 0600 after overnight fasting while allowing them to drink water freely after 2000 on the day before and we measured anthropological variables, arterial pressures, the carotid arterial compliance (CAC), and the baroreflex sensitivity (BRS) of heart rate (HR). On the third day of the week, the subjects returned to the laboratory at 1500 after finishing lunch before 1300 to undergo the measurement of peak aerobic capacity (VO$_{2peak}$) by a graded cycling exercise. On the fifth day of the week, they came to the laboratory at 0600 after overnight fasting, while allowing them to drink water freely after 2000 on the day before and we measured the plasma volume (PV) and blood constituents. Subjects then underwent the thermoregulatory response test. These measurements were repeated after training in the timeline shown in Fig. 1. After the pre-training measurements, we randomly divided subjects into two groups: glucose supplement (Glc; N=13) and whey-protein + glucose supplement (Pro-Glc; N=13) groups, so that there were no significant differences in the measurements, arterial blood pressures, and the medications (see details as below) between the groups but no subjects were aware of which group they belonged to.

The subjects in the Glc and Pro-Glc groups consumed a glucose supplement (25 g) and a mixture of whey-protein (10 g) + glucose supplement (15 g) dissolved in 200 ml water,
respectively, so that they were isocaloric, within 15 min after exercise each day. Subjects did not know which supplements they consumed. Then, they started cycle ergometer exercise training, 60 min·day⁻¹, 3 days·week⁻¹, for 8 weeks. After training, first, all subjects underwent the PV measurements and the thermoregulatory response test within 48-72 h after the last day of training before the training effects were lost. Then, they underwent other measurements but within 5 days. Subjects were instructed to take their medications regularly throughout the experimental period except for the morning of the day of the CAC and BRS measurements, and also the morning of the day of PV and blood constituent measurements and thermoregulatory response tests to avoid any acute effects on the results. Additionally, subjects were asked to refrain from ingesting alcohol and caffeine the day before and during the 5 days for the measurements.

Regarding the adherence to the training, two subjects in the Glc group dropped out of the training; one left because of a family issue and the other because of a health issue while in the Pro-Glc group, three subjects dropped out of training; one was due to a family issue and the other two were due to health issues. Therefore, we analyzed the results in 11 subjects in the Glc group and 10 subjects in the Pro-Glc group. The anthropological variables, the stage of hypertension, and the medications for the subjects were shown in Table 1. Other physical characteristics, PV and plasma constituents before and after training were shown in Table 2.

Exercise training:

Subjects in both groups came to the laboratory 0900-1100 or 1500-1700 and performed cycling exercise training for 60 min·day⁻¹, consisting of 4 sets of 15-min exercise and 5-min rest, 3 days·week⁻¹, interspersed with 1-2 no-exercise days, for 8 weeks. The exercise
intensity for the 1st week of training was 60% of the pre-training $\dot{V}O_{2\text{peak}}$, and it increased by 5% every week and to 75% of the pre-training $\dot{V}O_{2\text{peak}}$ by the end of the 4th week. After that, the intensity was adjusted every day so that the HR during the first 5 min of exercise was equivalent to 75% of the current $\dot{V}O_{2\text{peak}}$ which was estimated from the peak HR (HR_{peak}) at the pre-training $\dot{V}O_{2\text{peak}}$. $T_a$ and relative humidity (RH) in the training room were controlled at ~25 °C and ~50% with no significant differences between the groups. During daily exercise training, the subjects were allowed to drink tap water freely, and the amount of drinking water consumed was recorded. In addition, to estimate sweat loss during exercise, subjects were weighed in the nude after urination before exercise and just after exercise.

The criteria to stop exercise for safety were that HR was higher than 85% of the HR reserve, SBP/DBP were higher than 250/115 mmHg, or any abnormal electrocardiograph (ECG) measurements were observed (1). Accordingly, because arterial blood pressures increased more than the critical values, we reduced the exercise intensity by ~7% (~6 W) compared with the scheduled target level to maintain the training time per day in a subject for 16 days, 2 subjects for 4 days, and a subject for a day in the Glc group. Similarly, we reduced the intensity in a subject for 5 days and 2 subjects for a day in the Pro-Glc group. On the other hand, we observed no HR measurements reaching the critical value and no abnormal ECG results during the training period.

The amounts of drinking water consumed over the 8-week training period were 367 ± 73 (mean ± SE) and 337 ± 67 ml·day⁻¹, and the sweat loss was 840 ± 68 and 896 ± 108 g·day⁻¹ in the subjects who completed the training program in the Glc and Pro-Glc groups, respectively, with no significant differences between groups (both, P > 0.6).

The mean $T_a$ in Matsumoto was the highest in August at ~24.0 °C and lowest in January at ~ -0.6 °C, while the mean RH was ~65% throughout the period. No training was
conducted from the middle of July to the beginning of September to avoid any effects of living in a hot climate. Additionally, we paired subjects from the Glc and Pro-Glc groups, respectively, and had them to perform exercise training during the same days of the year with less than a 7 day lag period. During exercise training using the cycle ergometer, they were recommended to continue interval walking training as they did before.

The achievements of interval walking training by the subjects for 6 months before participating in the study were $2.2 \pm 0.5$ and $2.5 \pm 0.5$ days·week$^{-1}$ in the Glc and Pro-Glc group, respectively, and during the 2 months of the cycling training, they were $1.0 \pm 0.5$ and $0.6 \pm 0.3$ days·week$^{-1}$ in the Glc and Pro-Glc group, respectively, with no significant differences between groups (both, $P > 0.6$). In the present study, because we instructed subjects to perform cycling exercise at $60-75\% \dot{V}O_{2\text{peak}}$, $60 \text{ min} \cdot \text{day}^{-1}$, $3 \text{ days} \cdot \text{week}^{-1}$, during the training period, the volume of training (intensity x frequency x duration) were more than 4-fold higher than that before participating the study.

Supplements:

The glucose supplement was composed of 25 g glucose, 0 g protein, 0 g fat, and 0 mg sodium (Glucose, DHC, Tokyo, Japan). The whey-protein + glucose supplement was composed of 10 g whey-protein, 2.4 g maltose + trehalose, both of which were composed of 2 glucose molecules, 0g fat, 64 mg sodium (Savas Runner Protein, Meiji, Tokyo), and 12.5 g glucose (Glucose, DHC, Tokyo). The supplements were adjusted to ~100 kcal in both groups.

Dietary intake:

Subjects in both groups were instructed to maintain their dietary habits, except for the
supplements, during the study. They were not allowed to eat any food or drink any fluids except for water more than 120 min before and after exercise each training day, and they consumed the supplement assigned to each group after exercise. In addition, they were instructed to report food consumed for the consecutive 3 days for the 1st and the 8th training weeks, respectively, using a questionnaire. A dietitian calculated the daily nutrition intake with commercially available software (Excel Eiyokun, FFqg, Ver 3.0, Kenpakusya, Co. Ltd., Tokyo). As a result, without the supplements, 21 subjects in both groups consumed 1899 ± 56 kilocalories with diet; 254 ± 9 g carbohydrate, 46 ± 2 g protein, 54 ± 4 g fat, and 3864 ± 207 mg sodium per day with no significant differences between the groups (P > 0.18). These consumption levels met the recommended dietary allowances (RDA) for active, older Japanese men: total calories, 1850-2200 kcal·day^{-1}; carbohydrate, 231-385 g; protein, > 50 g·kg^{-1}·day^{-1}; fat, 41-61 g·day^{-1}; and sodium, < 3934 mg·day^{-1} (28).

Measurements:

Arterial blood pressures at rest

Before \( \dot{VO}_{2\text{peak}} \) measurement, we measured HR with an ECG (Life Scope 8; Nihon Kohden, Tokyo) and SBP and DBP by using the auscultation method after 10 min of rest from the right upper arm at the heart level by inflation of the cuff with sonometric pickup of Korotkoff’s sound (model STBP-780; Colin, Komaki, Japan) in the sitting position in an artificial chamber adjusted to 25 ± 0.1 °C (mean ± range) of \( T_a \) and 50 ± 1% of RH. The results were presented in Table 2.

\( \dot{VO}_{2\text{peak}} \)

On the same day after the measurements of arterial blood pressures at rest, we measured...
\( \dot{V}O_{2\text{peak}} \) using a cycle ergometer in an upright position in the artificial climate chamber at the environmental condition stated above. After measurements at rest for 3 min, subjects started pedaling at 60 revolutions \( \cdot \text{min}^{-1} \) without loading. The exercise intensity was increased by 30 W every 3 min until it reached 120 W and above this intensity, 15 W every 2 min until they could not maintain the rhythm due to exhaustion. We determined the \( \dot{V}O_2 \) every 15 s (Aeromonitor AE 260; Minato, Tokyo) and monitored ECG continuously during graded exercise. We determined \( \dot{V}O_{2\text{peak}} \) by averaging the three largest consecutive values at the end of exercise. During the measurements, we recorded HR with ECG, SBP, and DBP with the auscultation method every min. The criteria for determining \( \dot{V}O_{2\text{peak}} \) were that subjects could not keep the rhythm, the respiratory exchange ratio was > 1.1, and the HR reached the age-predicted maximum value. The HR_{peak} was adopted at the \( \dot{V}O_{2\text{peak}} \).

The criteria to stop exercise for safety reasons were as follows: the HR was higher than the age-predicted maximal HR, SBP/DBP were higher than 250/115 mmHg, or any abnormal ECG readings were observed (1). Accordingly, because arterial blood pressures increased more than the critical values, we stopped the test in subjects 3 and 1 before and after training, respectively, in the Glc group. Similarly, we stopped the tests, for subjects 5 and 4 before and after training, respectively, in the Pro-Glc group. In these cases, we used the values recorded at the stop but after confirming that HR_{peak} reached the age-predicted value and that the respiratory exchange ratio was > 1.1.

**PV and blood constituents**

On the day before the measurement, food was controlled over the course of the day (i.e., standardized breakfast, lunch, and dinner): total calories were ~2100 kcal, total carbohydrate was ~330 g, total protein ~67 g, total fat ~56 g, and sodium ~2.4 g before and after training.
in the Glc and Pro-Glc groups. Subjects were asked to eat the standardized breakfast and lunch at 0700 and 1200, respectively, and to finish the standardized dinner by 2100.

On the day of measurement, the subjects reported to the laboratory at 0600 normally hydrated but without having eaten any food for at least ~9 h before the measurement. To ensure that they were well hydrated, they were asked to drink ~500 ml water 2 h before the visit. After emptying their bladders, they were weighed and entered a room controlled to $T_a$ of ~28 °C and RH of ~50%. An 18-gauge Teflon catheter was then placed in the right antecubital vein for blood sampling and Evans blue dye injection. After subjects rested in a sitting position for 30 min, the PV was determined using the Evans blue dye dilution method (13, 38). Briefly, baseline blood samples were taken, the dye was injected, the blood samples were taken at 10 min after injection, and the absorbance (620 and 740 nm, U-1500; Hitachi, Tokyo) of a 10-min plasma sample was used to determine PV.

An aliquot of the baseline blood sample was transferred to a heparin treated tube and used to determine hematocrit (Hct, microcentrifuge) and hemoglobin concentration ([Hb], sodium lauryl sulfate hemoglobin method; Sigma Chemical, St Louis, MO) in triplicate. The remaining aliquot of sample was transferred to a heparin-treated tube and centrifuged at 4 °C for 30 min, and the separated plasma was used to determine the total plasma protein ([TP]$_p$) by refractometry and plasma albumin ([Alb]$_p$) concentrations by the bromcresol green method (Wako Chemical, Tokyo), osmolality ($P_{osm}$) by freezing-point depression (Fiske One-Ten osmometer, Needham Heights, MA), and plasma sodium concentration ([$Na^+$]$_p$) by flamephotometry (480 Flame Photometer; Corning, Medfield, MA). Total circulating plasma albumin content (Alb$_{cont}$) was calculated as a product of PV and [Alb]$_p$. The results are presented in Table 2 and the change in PV and Alb$_{cont}$ before and after training are presented in Fig. 2.
Thermoregulatory response test

After the PV measurements, the subjects were nude except for short pants and underwent the thermoregulatory response test at ~0830 on the same day. After emptying their bladders, they were weighed in the nude, and entered the environmentally controlled chamber at $T_a$ of 30.0 ± 0.1 °C (mean ± range) and RH 48.2 ± 0.4%. The 18-gauge Teflon catheter placed in the right antecubital vein for the PV measurement was used for blood sampling. The subjects rested quietly in a semirecumbent position in the contoured chair of the cycle ergometer for 60 min while all measurement devices were applied. Baseline measurements were taken for 10 min, and subjects performed cycling exercise in the semi-recumbent position at 60% of their pre-training $\dot{V}O_2$peak for 20 min without fan cooling. Blood samples were taken 10 min before and 2, 5, 10, 15 and 20 min after the start of exercise and used to determine Hct and [Hb] as described above. PV during the thermoregulatory response test was determined from the PV measured using the Evans blue dye dilution method, and the percent change in PV calculated from changes in Hct and [Hb] values (13) and the results are presented in Fig. 3. Throughout the test, we measured HR, SBP, DBP, $T_{es}$, mean skin temperature ($T_{sk}$), chest sweat rate (SR), and forearm skin blood flow (FBF) as described below.

$T_{es}$ was monitored with a thermocouple in a polyethylene tube (PE-90). The tube height into the esophagus was one-fourth of the subject’s standing height. $T_{sk}$ was monitored as $T_{sk} = 0.25T_{fa} + 0.43T_{ch} + 0.32T_{th}$ (40), where $T_{fa}$, $T_{ch}$ and $T_{th}$ are skin surface temperatures at the forearm 10 cm below the cubital line on the radial line, at the right chest 10 cm below the mid-clavicle, and at the right anterior thigh 10 cm above the patella on the middle line, which were measured with thermocouples, respectively. $T_{es}$ and $T_{sk}$ were recorded every 10
SR was determined by capacitance hygrometry, calculated from the RH and the temperature (THP-B3T; Shinee. Tokyo) of the air flowing out of a 12.56 cm² capsule at the rate of 1.5 l/min in the chest 5 cm below the left mid-clavicle. The FBF was measured by venous occlusion plethysmography with an indium-gallium-alloy-in-Silastic tube strain gauge placed around the upper side of the left forearm positioned above the heart level, with the hand eliminated from the circulation by inflating the occlusion cuff to supra-arterial pressure (280 mmHg) (48). The SR was recorded every 10 s, and the FBF was measured twice every minute, and they are presented every minute on average. In addition, the total sweat volume during 20 min of exercise was calculated based on changes in body weight before and after exercise. The measurements taken during the tests are shown only at rest, 5, and 20 min of exercise in Table 3 to avoid the complexity of the full table but the statistical analyses were performed by considering every minute.

HR, SBP, and DBP were measured every min. The mean blood pressure (MBP) was calculated as DBP + (SBP-DBP)/3. The results are shown in Fig. 4.

Forearm vascular conductance (FVC) was calculated as FBF/MBP (in ml·100 ml⁻¹·min⁻¹·100 mmHg⁻¹). The FVC and SR responses were shown in the left panels (A, B, C, and D) of Fig. 5 as a function of Tₑₛ. Because the increase in Tₑₛ was significantly lower after training than before training, we integrated changes in SR (Δ(∫ΔSR)dTₑₛ) and FVC (Δ(∫ΔFVC)dTₑₛ) above the baselines over the range of an increase in Tₑₛ after training and the difference in the areas under the curves of SR or FVC were compared between the Glc and Pro-Glc groups in the right panels (E and F) of Fig. 5.

The criteria to stop the test for the safety of the subjects were that Tₑₛ increased to over 38.5 °C in addition to the criteria for stopping exercise training as stated above.
Accordingly, we stopped the test in a subject for the Glc group at the 18th min of exercise before training, due to increased arterial blood pressures that were beyond the critical values. In that case, we used the values recorded at the time for the following analyses, assuming that the values remained unchanged for the last 2 min. On the other hand, we observed no HR and $T_e$ values exceeding the critical values and no abnormal ECG results during the test.

**CAC**

On the day of measurement, subjects reported to the laboratory at 0600 normally hydrated. To ensure that they were well hydrated, they were asked to drink ~200 ml water after getting out of bed. After emptying their bladders, they were weighed and entered an environmental chamber controlled to ~28 °C of $T_a$ and ~40% of RH, and rested in a supine position for more than 30 min before the measurements. We analyzed the 10/11 subjects in the Glc group and 9/10 subjects in the Pro-Glc group because 2 subjects quit the measurements due to urination.

An Echo-Doppler ultrasound imaging system (Vivid 7, General Electric, Fairfield, CT, USA) equipped with a high resolution liner array transducer (14.0 MHz) was used to determine the diameter of the common carotid artery. Images of the left common carotid artery were collected at ~14 frames per second (sampling rate = 14.1 Hz) for 5 cardiac cycles during each of the 10-min measurement periods (Fig. 6 A). The sonographer acquired longitudinal images of the distal common carotid artery 1 cm proximal to the carotid bulb when the interfaces of both the near and far wall were clearly visualized by placing the transducer perpendicular to the vessel wall. To determine the diameter, the perpendicular distance from the adventitial-medial interface on the near wall to the medial-adventitial interface on the far wall was measured for every frame (46). The amplitudes of the change in
the diameter with heart cycle between the peak and bottom values were averaged for 5 heartbeats. The same investigator, who was not aware of which group the subjects belonged to, performed all the measurement to minimize the variation due to inter-individual technical difference. Pulsatile arterial blood pressure synchronized with the diameter change of the common arterial pressure was determined by finger photoplethysmography (Finometor; Finapres Medical Systems, Amsterdam, the Netherlands) using a volume clamp method. HR was determined by five-lead ECG from R-R intervals.

Compliance was calculated according to the following formula (24),

\[ C = \frac{(D_1-D_0)/D_0}{2(P_1-P_0)} \times \pi \times D_0^2, \]

where \( P_0 \) is diastolic pressure and \( P_1 \) is systolic pressure. \( D_0 \) is the smallest diameter in response to \( P_0 \) and \( D_1 \) is the maximal diameter in response to \( P_1 \). The results before and after training are shown in Fig. 6 C.

**BRS**

After determining CAC, we determined the BRS in the supine position from HR response to change in arterial blood pressure during the Valsalva maneuver (Fig. 6 B). For the maneuver, subjects held their breath for 10 sec so that the expiratory pressure was increased and maintained at 40 mmHg, which was monitored with a pressure transducer placed in the mouth, in a feedback manner while watching the current pressure displayed visually. Then, they released their breath holding and breathed regularly until HR and arterial pressure returned to their baseline values. They repeated 3 sets of this procedure with 3-min rests between tests, and the average results were used.

Briefly, the Valsalva maneuver consists of 4 phases lasting for several seconds each; 1) before, 2) during, 3) immediately after, and 4) late after the breath holding (44). The BRS
was determined from a decrease in HR (ΔHR) in response to an increase in SBP (ΔSBP) as
ΔHR/ΔSBP during the 4th phase of the Valsalva maneuver after correcting for the latency of
the HR decrease after the SBP increase (8). The latency was 3.3 ± 0.4 sec and 4.5 ± 0.5 sec
before training and 3.6 ± 0.5 sec and 4.2 ± 1.2 sec after training in the Glc and Pro-Glc
group, respectively, with no significant differences between before and after training (P >
0.9) or between groups (P > 0.2). The results were shown in Fig. 6 D.

Statistics:

One-way ANOVA was used to examine significant differences in the anthropological
variables, and the Mann-Whitney U-test was used to examine significant differences in the
stage of hypertension, and the medications between groups (Table 1). Two-way [1
between groups and 1 within training] (the latency for Valsalva maneuver, Table 2, Fig. 2 A
and B, Fig. 5 E and F, Fig. 6 C and D) or three-way [1 between groups and 2 within training
and time] ANOVA (Table 3, Figs. 3 and 4) for repeated measures were used to examine the
significant effects of group (Glc vs. Pro-Glc), training (before vs. after), and time during the
thermoregulatory response test on the variables. Additionally, to examine the significantly
different effects of training between the groups, we determined the interactive effects of
[group × training] on the variables (Tables 2 and 3, Figs. 2-4, Fig. 5 E and F, Fig. 6 C and
D). After confirming significant differences by ANOVA, a subsequent post hoc test was
performed to examine significant differences in various pairwise comparisons using the
Turkey-Kramer test. The null hypothesis was rejected when P < 0.05 (41).

RESULTS

Table 1 shows the anthropological variables, the hypertension classification, and the
medication before training. There were no significant differences in the age, height, and BMI between groups (all, P > 0.2). According to the guideline released by the AHA (6), 0, 7, and 4 of the 11 subjects for the Glc group and 1, 3, and 6 of the 10 subjects for the Pro-Glc group belonged to the categories of prehypertension, stage 1 HTN and stage 2 HTN, respectively. When 0, 1, and 2 points were assigned to the respective categories, the averaged value was 1.4 ± 0.2 in the Glc group and 1.5 ± 0.2 in the Pro-Glc group with no significant difference between them (P = 0.45). Similarly, on average, the number of drugs subjects used was 1.6 ± 2.3 and 1.9 ± 2.2 in the Glc and Pro-Glc groups, respectively, with no significant difference between them (P = 0.91).

Table 2 shows the physical characteristics of subjects before and after training. Before training, there were no significant differences in any variables between groups (P = 0.25-0.75). After training, the DBP and MBP decreased and the $\dot{V}O_2$peak increased significantly but with no interactive effects of [training x group] on the variables (all, P > 0.4). The table also shows PV and plasma constituents at rest prior to the thermoregulatory response test before and after training. After training, PV and Albcont significantly increased in the Pro-Glc group (P < 0.0001 and P = 0.0003, respectively) but not in the Glc group (P = 0.073 and P = 0.69, respectively), with significant interactive effects of [training x group] on the PV and Albcont, suggesting that the whey protein + glucose supplement enhanced the increase in PV and Albcont.

Accordingly, we analyzed the changes in PV after training in the Glc and Pro-Glc groups. As in Fig. 2 A and B, after training, PV and Albcont in the Pro-Glc group increased by 6.6 % and 5.4 %, respectively (both, P < 0.001). We confirmed that the increases in PV and Albcont by training were significantly greater in the Pro-Glc group than in the Glc group with significant interactive effects of [training x group] on PV (P = 0.022) and Albcont (P =
Furthermore, we found that the increase in PV was highly correlated with the increase in Alb_{cont} (R^2 = 0.70, P < 0.001).

**Fig. 3** shows the profiles of PV during the thermoregulatory response test. As in the figure, PV during exercise significantly increased after training in the Glc and Pro-Glc groups (P = 0.019 and P < 0.001, respectively). The increase was marginal but not significantly higher in the Pro-Glc group than in the Glc group (P=0.055).

**Fig. 4** shows the profiles of HR, SBP, DBP, and MBP during the thermoregulatory response test in the Glc and Pro-Glc groups. These values at rest and during exercise significantly decreased in both groups except for SBP at rest in the Glc group, with no significant interactive effects of [training x group] on the variables (all, P > 0.3).

**Table 3** shows T_{es}, T_{sk}, SR and FBF during the thermoregulatory response test. After training, an increase in T_{es} during exercise was significantly attenuated in the Pro-Glc group (P = 0.0012) but not in the Glc group (P = 0.086), with no significant interactive effect of [training x group] on T_{es} (P = 0.22).

**Fig. 5 A-D** shows SR and FVC responses to T_{es} during the thermoregulatory response test in the Glc and Pro-Glc groups. **Fig. 5 E & F** shows the difference in the areas under the curves of SR (∫[ΔSR]dT_{es}) or FVC (∫[ΔFVC]dT_{es}) in the left panels between before and after training. As in **Fig. 5 A-D**, the SR and FVC responses appeared to be more enhanced after training in the Pro-Glc group than in the Glc group. SR increased in 8/10 subjects for the Pro-Glc group, while it increased in 6/11 subjects for the Glc group. On the other hand, FVC increased in 10/10 subjects in the Pro-Glc group but only in 5/11 subjects in the Glc group. As a result, a significant increase in FVC from the baseline was observed only in FVC (P < 0.0001), with a significant interactive effect of [training x group] on FVC (P = 0.006; **Fig. 5 F**).
The Fig. 6 C & D shows CAC and ΔHR/ΔSBP before and after training in the Glc and Pro-Glc groups. As in the figure, after training, CAC increased by 21.3% in the Pro-Glc group (P = 0.031), but it remained unchanged in the Glc group (P = 0.21), with no significant interactive effect of [training × group] (P = 0.39). Additionally, after training, ΔHR/ΔSBP increased by 66% and 132% in the Glc and Pro-Glc groups, respectively, (P = 0.036 and P = 0.009, respectively) but with no significant interactive effect of [training × group] (P = 0.74).

To examine any effects of anti-hypertensive drugs on the results, we analyzed the results in the subjects with no anti-hypertensive medications: 7/11 and 6/10 subjects in the Glc and Pro-Glc groups, respectively (Table 1). We confirmed a significant interactive effect of [training x group] on the increases in PV and Alb\textsubscript{cont} at rest (P = 0.014 and P = 0.025, respectively) and on FVC responses to increased T\textsubscript{es} during the thermoregulatory response test (P = 0.008). In addition, we confirmed that HR, SBP, DBP, and MBP during the thermoregulatory response test significantly decreased after training in the Glc and Pro-Glc groups (P = 0.026 and P = 0.034, respectively). Finally, we confirmed that ΔHR/ΔSBP increased significantly after training in the Pro-Glc group (P = 0.010) but not in the Glc group (P = 0.17).

DISCUSSION

In the present study, we studied the older men with higher arterial blood pressure than those in the previous study (37), and those with hypertension matched the categories of the guidelines of the AHA (6). The major findings in the present study are that 1) the cutaneous vasodilator response to increased T\textsubscript{es} during exercise was enhanced with increased PV in the Pro-Glc group but not in the Glc group; 2) despite the increase in PV for the Pro-Glc group,
arterial blood pressures did not increase; rather, it decreased as in the Glc group; and 3) the 
sensitivity of baroreflex control of the HR increased in both groups, with a significant 
increase in CAC in the Pro-Glc group but not in the Glc group.

According to the report published by the Ministry of Environment of Japan in 2014 (27), 
the number of days in which the highest atmospheric temperature during the midsummer 
was over 30 °C had been ~40 days per year before 2000, but thereafter, it has gradually 
increased to more than 140 days per year in 2014. Accordingly, they reported that the 
number of patients transported to hospitals due to heat illness was ~50,000 per year on 
average for the last decade, and ~800 of them died per year. More importantly for the 
present study, 47% of the patients were over 65 years old, and they shared 77% of the deaths 
in 2013 (27). Thus, the countermeasures against heat illness for older people have been 
awaited.

Recently, Okazaki et al. (37) suggested that whey-protein + carbohydrate 
supplementation during aerobic training for 8 weeks increased thermoregulatory responses 
with PV expansion of ~6%, whereas placebo intake did not cause such expansion in 
normotensive older men. As for the mechanisms, they suggested that albumin synthesis was 
reportedly enhanced in the liver immediately after intense exercise (31) and that the timing 
of ingesting a mixture of whey-protein + carbohydrate allowed it to be effectively used for 
the synthesis. Because the synthesized albumin was released into the systemic circulation, it 
increased colloid osmotic pressure in the plasma to cause a fluid shift from the extra- to 
intravascular space through the capillary area to increase PV, which in turn increased 
cutaneous vasodilation and sweat rate responses to an increase in T_{es} during exercise as 
reported in young people before (11, 19). Thus, the prescription may be one of the 
countermeasures against heat illness for older people.
On the other hand, it has been reported that more than 60% of older men in their 60s and more than 70% of those in their 70s suffer from hypertension in Japan (17) and the US (30), and an increase in PV might be a risk factor that worsens the symptoms of hypertension by increasing the mean circulatory filling pressure due to decreased total vascular compliance, and increasing venous rerun to the heart/ cardiac output, resulting in the renal adaptation and an upward shift of the set point of arterial pressure. These are reasons why the prescription has not been recommended broadly such as by clinicians to prevent heat illness in older people. Thus, there is an apparent trade-off between thermal and arterial blood pressure regulation in older people. The present study was conducted to solve this.

Subjects:

As in Table 2, SBP and DBP in the subjects in the present study were ~160 mmHg and ~90 mmHg on average, respectively, higher than ~120 mmHg and ~80 mmHg in the previous study (37). These values matched the criteria for hypertension according to the AHA guidelines (6), as is true for more than 60% of older people suffered from (17, 30). In addition, the VO\textsubscript{2peak} of the subjects in the present study was ~28 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} on average, lower than ~35 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} in the previous study (37) but equal to the average values for the Japanese (23) and American men (18) of the same age. These results suggest that the subjects in the present study were likely more representatives for the men of the age group than in the previous study (37). Therefore, if we obtained the results to support the hypotheses, the prescription would be accepted by a large population of older people to prevent heat illness.
PV and Alb_{cont}:

As in Fig. 2, PV and Alb_{cont} increased in the Pro-Glc group but not in the Glc group. Because ΔPV at rest was highly correlated with ΔAlb_{cont}, the increase in PV was likely caused by an increase in Alb_{cont} as suggested before in older subjects (37) and young subjects (11, 19). In addition, unlike in the previous study (37), in the present study, we used glucose as a placebo for the Glc group, adjusted to be iso-caloric with the supplement for the Pro-Glc group, indicating that whey-protein supplementation was necessary to cause PV expansion in this protocol. Thus, we reconfirmed the merits of a mixture of whey-protein + glucose intake immediately after exercise for aerobic training to increase PV in older subjects with higher arterial blood pressures than in the previous study (37). On the other hand, the mechanisms for the marginal increase in PV in the Glc group (Figs 2 and 3) remained unknown; however, extracellular fluid expansion due to accelerated renal Na^+ reabsorption with enhanced insulin secretion by glucose intake might be involved (21).

Thermo-regulatory response:

As in Fig. 5, we found that the FVC response to increased T_{es} was significantly enhanced after training in the Pro-Glc group but not in the Glc group. In our previous study (37), we analyzed the FVC response to increased T_{es} during exercise at a similar relative intensity in the similar environmental conditions after aerobic training before and after aerobic training as in the present study, and we suggested that the sensitivities of FVC response to a given increase in T_{es} increased by 80% when PV increased by ~6% in the whey-protein + carbohydrate supplement group but not in the placebo group, in which PV almost remained unchanged. Although, in the previous study, T_{es} at rest was not significantly different before and after training in both groups, in the present study, T_{es} at rest
and at the end of exercise decreased by ~0.1 °C and ~0.3 °C, respectively, after training in the Pro-Glc group (Table 3). Therefore, we calculated the difference of the area under the curve of the FVC response in the range of Tes variation commonly observed before and after training in each group instead of using the traditional method to evaluate the FVC response (37). As a result, we found that a mixture of whey-protein + glucose supplementation during aerobic training significantly improved FVC responses in older men with higher arterial blood pressures than in the previous study (37).

Regarding the mechanisms of the improved FVC response in the Pro-Glc group, it has been suggested that acute hypervolemia by blood transfusion (9) and saline infusion (36), and an increase in venous return to the heart by changing posture from the upright to supine position (10, 20), by negative pressure breathing (32), and by head-out water immersion (35), enhanced the cutaneous vasodilation during exercise though in young subjects. In the previous study (37), we suggested that the same mechanisms worked in the whey-protein + carbohydrate supplement group because cardiac stroke volume significantly increased in the treatment group but not in the placebo group. Although we did not measure cardiac stroke volume in the present study, we confirmed that PV after training maintained a significantly higher level than before training during the thermoregulatory response test (Fig. 3), and the increase was marginally greater in the Pro-Glc than in the Glc group (P = 0.055). These results support the idea that stretching the cardiopulmonary mechanoreceptors with an increase in venous return to the heart enhances cutaneous vasodilation in the older men with higher arterial blood pressure than in the previous study (37).

On the other hand, although the SR response to increased Tes was significantly enhanced by 18% in the Pro-Glc group in the previous study (37), in the present study, we found no significant improvement of the response, calculated similarly to the FVC response, after
training in the Pro-Glc group, despite the trend shown in Fig. 5 C, which was likely due to higher inter-individual variation of the response than in the previous study (37).

Arterial blood pressures:

As in Fig. 4, HR and DBP were significantly lower at rest and during exercise after training in both groups; however, more importantly, in the present study, after training, SBP and DBP during exercise decreased in the Pro-Glc group by a similar degree to the Glc group despite the significant increase in PV.

It has been suggested that aerobic exercise training decreases arterial blood pressure by decreasing arterial wall stiffness (46) and sympathetic nervous out flow (4, 12, 25), and by improving BRS (25) in middle-aged and older people with hypertension, though after more prolonged exercise training than in the present study. In the present study, BRS increased by 66% in the Glc group and by 132% in the Pro-Glc group, and CAC significantly increased by 21% in the Pro-Glc group (Fig. 6), but we did not measure sympathetic nervous activity. As a result, we found that MBP decreased by ~10 mmHg at rest and ~20 mmHg during exercise after training in both groups, equivalent to ~10% and ~14% lower than the pre-training values. On the other hand, because the increase in PV in the Pro-Glc group was only ~6% of the pre-training value, a possible increase in cardiac stroke volume/ cardiac output expected from the increase in PV was much less than the decrease in MBP as experimentally observed. These results suggest that the mechanisms for decreasing arterial blood pressure by aerobic training were strong enough to buffer a possible increase in arterial pressure by increased PV. However, importantly in the present study, we found that even such a small increase in PV caused a significant improvement in cutaneous vasodilation in older men with higher arterial blood pressures than in the previous study (37)
The detailed mechanisms for the increased CAC in the Pro-Glc group remain unknown. However, the arterial compliance decreases with advancing age, which was reportedly partially caused by decreased elastin in the vascular wall (5), a phenomenon that is a greater risk in hypertensive patients (39) and animal models (2, 3, 7, 45). The use of whey-protein in the supplement for the Pro-Glc group adds relatively large amounts of amino acids, which could contribute to elastin turnover. Insulin stimulated by glucose in the supplement may work advantageously to incorporate the amino acids into the elastin synthesis in the vascular wall. Alternatively, it has been suggested that mitochondrial dysfunction due to muscle atrophy with aging causes chronic inflammation in the body (16), and, if it occurs in the immune cells in the vascular wall, it causes atherosclerosis (42). Recently we found that NFκB (nuclear factor-kappa B) genes in the white cells, the master genes of inflammatory reactions, were inactivated by milk product supplementation during walking training (26). These results suggest that a mixture of whey-protein + glucose supplementation during aerobic training increases arterial compliance by increasing the elasticity of the vascular wall and/or by suppressing chronic inflammation in the endothelium of the arterial wall.

Effects of medication:

We designed the present study to exclude any effects of medication, not only with anti-hypertensive drugs but also with other drugs such as for dyslipidemia and/or diabetes mellitus, on the results as much as possible. We confirmed no significant differences in the medications between groups (Table 1), and when we analyzed the results in the subjects with no medication with hypertensive drugs, we found the similar results as in the analysis of all subjects in terms of the changes in PV, Albcont, FVC responses to increased $T_{es}$, arterial
blood pressures at rest and during exercise, and in BRS after training. Thus, the effects of medication on the results would be minimal, if any, irrespective of other drugs for co-morbidities.

General foods for supplement:

In the present study, we used a mixture of ~10 g whey-protein + ~15g glucose as a supplement to examine the effects on Alb_{cont} in older people with hypertension. Since it has been suggested that the protein synthesis in the skeletal muscle was enhanced with casein-intake similarly as with whey-protein intake when they were consumed immediately after exercise (39, 47), any milk products may also enhance the albumin synthesis in the liver at the timing. For example, ~200 ml milk contains ~5.3 g casein-protein and ~1.3 g whey-protein with ~10 g carbohydrate, and ~40 g cheese contains ~9.1 g casein-protein with minimal carbohydrate. Therefore, it may be recommended to consume them together with foods containing some carbohydrate at the timing.

In conclusion, a mixture of whey-protein and glucose supplementation immediately after exercise during aerobic training increased PV and thereby improved cutaneous vasodilation during exercise in a warm environment while arterial blood pressures did not increase but rather decreased in older men with higher arterial blood pressure than the previous study (37). This suggests that the prescription is one of the effective countermeasures for a large population of older people, even for those who suffer from hypertension, to help prevent heat illness during exercise in the midsummer.

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Sports Medical Sciences, Shinshu University Graduate School of Medicine for their technical assistance.

GRANTS

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DISCLOSURE

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


2. Boumaza S, Arribas SM, Osborne-Pellegrin M, McGrath JC, Laurent S, Lacolley P, Challande P. Fenestrations of the carotid internal elastic lamina and


FIGURE LEGENDS:

Fig. 1:
A timeline of the assessments for 5 days before and after exercise training. CAC, carotid arterial compliance; BRS, baroreflex sensitivity of heart rate; \( \dot{\text{VO}}_2\text{peak} \), peak aerobic capacity, PV, plasma volume; T.R. test, thermoregulatory response test.

Fig. 2:
A: Changes in plasma volume (\( \Delta \text{PV} \)) and B: plasma albumin content (\( \Delta \text{Alb}_{\text{cont}} \)) from the baselines after training for 8 weeks in the glucose alone (Glc) and whey-protein + glucose supplement (Pro-Glc) groups. Values are means ± SE for 11 and 10 subjects in the Glc and Pro-Glc groups, respectively. ***, P < 0.001 vs. before training. †, P < 0.05 between groups.

Fig. 3:
Plasma volume (PV) during the thermoregulatory response test before (open symbols) and after (closed symbols) 8-week training in the Glc and Pro-Glc groups. PV; plasma volume. Values are means ± SE for 11 and 10 subjects in the Glc and Pro-Glc groups. *, P < 0.05 vs. before training.

Fig. 4:
Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP) during the thermoregulatory response test before (open symbols) and after (closed symbols) 8-week training in the Glc and Pro-Glc groups. Values are means ± SE for 11 and 10 subjects in the Glc and Pro-Glc groups, respectively. *, P < 0.05 vs. before training.
Fig. 5:

Left panels: Sweat rate (SR: A & C) and forearm vascular conductance (FVC: B & D) responses to increased esophageal temperature (T_es) during the thermoregulatory response test in the Glc (A & B) and Pro-Glc groups (C & D) before (open symbols) and after (closed symbols) 8-week training. Right panels: The differences in the areas under the curves of SR (E) and FVC (F) between before and after 8-week training in the Glc (A & B) and Pro-Glc (C & D) groups, respectively. Values are means ± SE for 11 and 10 subjects in the Glc and Pro-Glc groups, respectively. ***, P < 0.001 vs. before training. ††, P < 0.01 between groups.

Fig. 6:

A: Typical examples of an electrocardiogram (ECG), common carotid arterial diameter (CCAD), and arterial pressure (AP) to determine carotid arterial compliance. B: Changes in systolic blood pressure (ΔSBP) and heart rate (ΔHR) during the Valsalva maneuver to determine baroreflex sensitivity of HR. IOP, intra-oral pressure. C: Carotid arterial compliance (CAC) is shown as means ± SE bars for 10 and 9 subjects before (open columns) and after (closed columns) 8-week training in the Glc and Pro-Glc groups, respectively. D: Similarly, baroreflex sensitivity of HR (ΔHR/ΔSBP) is shown as means ± SE bars for 10 and 9 subjects before (open columns) and after (closed columns) 8-week training in the Glc and Pro-Glc groups, respectively. *, P < 0.05; **, P < 0.01 vs. before training.
### Table 1: Age, height, BMI, hypertension classification, and medication of anti-hypertensive drugs and other drugs in the Glc and Pro-Glc groups

<table>
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<tr>
<th>Subjects #</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>BMI, kg·m²</th>
<th>H.C.</th>
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<th>Other drugs</th>
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<td>160</td>
<td>21</td>
<td>S2</td>
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<tr>
<td>2</td>
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<td>164</td>
<td>23</td>
<td>S1</td>
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<td><strong>22 ± 1</strong></td>
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Four of 11 subjects in the Glc group and 4 of 10 subjects in the Pro-Glc group were medicated with anti-hypertensives. H.C., hypertension classification according to the Am. Heart Assn. (6); S1, Stage 1 hypertension; S2, Stage 2 hypertension; P.H., Prehypertension.
Table 2: Physical characteristics, plasma volume and constituents before and after training

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<td>After</td>
<td>Before</td>
<td>After</td>
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<td>Body mass, kg</td>
<td>60.9 ± 2.2</td>
<td>60.9 ± 2.2</td>
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<td>81 ± 3 **</td>
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<td>114 ± 3</td>
<td>107 ± 2 *</td>
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<td>( \dot{\text{VO}}_2\text{peak}, \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} )</td>
<td>27.4 ± 1.1</td>
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<td>28.2 ± 1.5</td>
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<td>1.9 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1 ***</td>
<td>0.005</td>
<td>NS</td>
</tr>
<tr>
<td>(\text{P}_{\text{osm}}, \text{mosmol·kgH}_2\text{O}^{-1})</td>
<td>292 ± 1</td>
<td>290 ± 1</td>
<td>292 ± 1</td>
<td>291 ± 1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>([\text{Na}^+]_p), meq·kgH_2O(^{-1})</td>
<td>150 ± 1</td>
<td>150 ± 1</td>
<td>151 ± 1</td>
<td>150 ± 1</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean ± SE for Glc, glucose supplement, and Pro-Glc, whey protein + glucose supplement groups; SBP, DBP, and MBP, systolic, diastolic, and mean blood pressures, respectively; \( \dot{\text{VO}}_2\text{peak} \), peak aerobic capacity for cycling; HR\(_{\text{peak}} \), peak heart rate at \( \dot{\text{VO}}_2\text{peak} \); PV, plasma volume; \([\text{TP}]_p\), total plasma protein concentration; \([\text{Alb}]_p\), plasma albumin concentration; Alb\(_{\text{cont}}\), albumin content; \(\text{P}_{\text{osm}}\), plasma osmolality; \([\text{Na}^+]_p\), plasma sodium concentration; Tr, training (before vs. after); G, groups (Glc vs. Pro-Glc); Tr x G, interactive effects of training and group. *, **, *** Significant differences vs. before training in each group at P < 0.05, P < 0.01, and P < 0.001, respectively.
Table 3: $T_{es}$, $T_{sk}$, SR, and FBF during thermoregulatory response test before and after training

<table>
<thead>
<tr>
<th></th>
<th>Glc (N = 11)</th>
<th>Pro-Glc (N = 10)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>5 min</td>
<td>20 min</td>
</tr>
<tr>
<td>$T_{es}$, °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>36.9 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.9 ± 0.1</td>
</tr>
<tr>
<td>After</td>
<td>36.9 ± 0.1</td>
<td>36.9 ± 0.1</td>
<td>37.7 ± 0.1</td>
</tr>
<tr>
<td>$T_{sk}$, °C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>33.8 ± 0.1</td>
<td>33.5 ± 0.1</td>
<td>33.4 ± 0.1</td>
</tr>
<tr>
<td>After</td>
<td>33.8 ± 0.2</td>
<td>33.6 ± 0.2</td>
<td>33.3 ± 0.2</td>
</tr>
<tr>
<td>SR, mg·cm$^{-2}$·min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>0.00 ± 0.00</td>
<td>0.03 ± 0.02</td>
<td>0.51 ± 0.14</td>
</tr>
<tr>
<td>After</td>
<td>0.00 ± 0.00</td>
<td>0.01 ± 0.01</td>
<td>0.50 ± 0.13</td>
</tr>
<tr>
<td>FBF, ml·100 ml$^{-1}$·min$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>3.84 ± 0.37</td>
<td>4.54 ± 0.50</td>
<td>7.93 ± 0.55</td>
</tr>
<tr>
<td>After</td>
<td>3.80 ± 0.21</td>
<td>4.29 ± 0.62</td>
<td>9.09 ± 0.85</td>
</tr>
</tbody>
</table>

Values are means ± SE for Glc, glucose supplement, and Pro-Glc, whey-protein + glucose supplement groups; $T_{es}$, esophageal temperature; $T_{sk}$, mean skin temperature; SR, sweat rate; FBF, forearm skin blood flow; Tr, training (before vs. after); G, groups (Glc vs. Pro-Glc); Tr x G, interactive effects of training and group. **, Significant differences vs. before training at P < 0.01.
A

$\Delta$PV, ml $\cdot$ kg$^{-1}$

B

$\Delta$Alb$_{cont}$, g $\cdot$ kg$^{-1}$

Kataoka et al., Fig. 2
Glc

Pro-Glc

HR, bpm

SBP, mmHg

DBP, mmHg

MBP, mmHg

Time, min

Kataoka et al., Fig. 4
Kataoka et al., Fig. 5