Protein and carbohydrate supplementation during 5-day aerobic training enhanced plasma volume expansion and thermoregulatory adaptation in young men

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Goto M, Okazaki K, Kamijo Y, Ikegawa S, Masuki S, Miyagawa K, Nose H. Protein and carbohydrate supplementation during 5-day aerobic training enhanced plasma volume expansion and thermoregulatory adaptation in young men. J Appl Physiol 109: 1247–1255, 2010. First published August 5, 2010; doi:10.1152/japplphysiol.00577.2010.—We examined whether protein and carbohydrate (CHO) supplementation during 5-day training enhanced plasma volume (PV) expansion and thermoregulatory and cardiovascular adaptations in young men. Eighteen men (age 23 ± 4 SD) were divided into two groups according to supplements: placebo (CNT: 0.93 kcal/kg, 0.01 g protein/kg, n = 9) and protein and CHO (Pro-CHO: 3.6 kcal/kg, 0.36 protein/kg, n = 9). Subjects in both groups performed a cycling exercise at 70% peak oxygen consumption rate (\(\dot{V}\)O\(_{2\text{peak}}\)) 30 min/day for 5 consecutive days at 30°C ambient temperature and 50% relative humidity and took either a placebo or Pro-CHO within 10 min after exercise for each day. Before and after training, PV at rest, heart rate (HR), and esophageal temperature (T\(_{es}\)) during 30-min exercise at 65% of pretraining \(\dot{V}\)O\(_{2\text{peak}}\) in the same condition as training were determined. Also, the sensitivity of the chest sweat rate (\(\Delta\)SR/\(\Delta\)T\(_{es}\)) and forearm vascular conductance (\(\Delta\)FVC/\(\Delta\)T\(_{es}\)) in response to increased T\(_{es}\) were determined. After training, PV and cardiac stroke volume (SV) at rest increased in both groups (P < 0.001) but the increases were twofold higher in Pro-CHO than CNT (P = 0.007 and P = 0.078, respectively). The increases in HR from 5 to 30 min and T\(_{es}\) from 0 to 30 min of exercise were attenuated after training in both groups with greater attenuation in Pro-CHO than CNT (P = 0.002 and P = 0.072, respectively). \(\Delta\)SR/\(\Delta\)T\(_{es}\) increased in CNT (P = 0.052) and Pro-CHO (P < 0.001) and the increases were higher in Pro-CHO than CNT (P = 0.018). \(\Delta\)FVC/\(\Delta\)T\(_{es}\) increased in Pro-CHO (P < 0.001), whereas not in CNT (P = 0.16). Thus protein-CHO supplementation during 5-day training enhanced PV expansion and thermoregulatory adaptation and, thereby, the reduction in heat and cardiovascular strain in young men.

HIGHLIGHTED TOPIC | Mechanisms and Modulators of Temperature Regulation

Protein and carbohydrate supplementation during 5-day aerobic training enhanced plasma volume expansion and thermoregulatory adaptation in young men.
ment, no subject was taking medication that would influence cardiovascular or thermoregulatory function, blood volume, and blood constituents.

Protocol

Two days after the \( V_{\text{O}_2}\text{peak} \) determination, subjects underwent PV measurement and a thermoregulatory response test on the same day, as described below. Subjects were then divided into two groups: 1) protein and CHO mixture (Pro-CHO; \( n = 9 \)) and 2) non-protein and low-calorie placebo (CNT; \( n = 9 \)) so as to have no significant differences in anthropometric measures, PV, \( V_{\text{O}_2}\text{peak} \), and blood constituents between groups (Tables 1 and 2).

Two days after the PV measurement and thermoregulatory response test, subjects in both groups carried out 5 consecutive days of aerobic training, as described below, and were given their respective supplement immediately after each bout of exercise. All subjects and investigators were blinded to the subject groups. All experiments were performed between October and June to avoid any effect of heat acclimatization in the summer season. Average atmospheric temperature \((T_a)\) was \(-1^\circ\text{C}\) in January and \(19^\circ\text{C}\) in June. Average relative humidity \((\text{RH})\) was 60–76%.

Within 36 to 42 h after the termination of training, the PV measurement and the thermoregulatory response test were performed again on the same day. \( V_{\text{O}_2}\text{peak} \) was determined within 24 to 48 h after the test. All measurements and tests, except for \( V_{\text{O}_2}\text{peak} \), were performed at the same time in the morning to avoid any effect of circadian variations.

Aerobic Training Regimen

Aerobic training was performed at 1400–1800 and between lunch and dinner more than 2 h after and before meals in an environmental chamber controlled to 30.0 ± 0.1°C \( T_a \) and 50 ± 1% RH \((\text{mean} \pm \text{range})\). Subjects exercised on a cycle ergometer in an upright position at 70% of pretraining \( V_{\text{O}_2}\text{peak} \) for 30 min/day for 5 days. \( H_R \) was continuously monitored during exercise, and the exercise intensity was readjusted 5 min after the start of exercise each day so that subjects exercised at a target heart rate \((H_R)\) equivalent to 70% \( V_{\text{O}_2}\text{peak} \)-determined from the relationship between the oxygen consumption rate \((\text{O}_2)\) and \( H_R \) at the \( V_{\text{O}_2}\text{peak} \) measurement before training. The reason for adopting the \( H_R \) at 5 min as the target \( H_R \) to be readjusted was that it depended on the relative exercise intensity before the body temperature started to increase. During exercise, subjects were not allowed to drink any fluid before taking supplements. Sweat loss was estimated from body weight loss after exercise: 635 ± 68 and 643 ± 64 g/day in CNT and Pro-CHO, respectively, over the 5-day training period, with no significant differences between groups \((P = 0.93)\).

We chose this training protocol because exercise stress as well as thermal stimulation was suggested to significantly contribute to aerobic training-induced PV expansion and thermoregulatory response enhancement (3) and also because protein and CHO supplementation immediately after a bout of intense exercise evoked greater increases in PV and \( Alb_{\text{con}} \) in our previous study, and we surmised that high exercise intensity as well as high thermal stimulation would be needed to increase PV and improve thermoregulatory responses during training. Thirty minutes of exercise per day was thought to be the upper limit for subjects to continue to exercise at this intensity with a low risk for heat stroke. The reason for choosing a 5-day training period was that this is reportedly sufficient for most reductions of HR and core temperature during exercise in a warm environment in young people (17).

Supplements

Subjects ingested 6.4 ml/kg of a protein and CHO mixture (56 kcal, 8.3 g CHO, and 5.6 g protein/100 ml) containing 3.6 kcal/kg, 0.47 g CHO/kg, 0.36 g protein/kg in the Pro-CHO group or the same volume of a non-protein and low-calorie placebo (15 kcal, 1.7 g CHO, and 0 g protein/100 ml) containing 0.93 kcal/kg, 0.09 g CHO/kg, 0 g protein/kg in the CNT group within 10 min after exercise each day. Sodium intake from the supplements was 1.0 mg/kg in both groups. The amount of protein intake by supplementation in Pro-CHO was determined so that it was equivalent to ~20% of daily protein intake according to a previous study on older subjects (21).

Dietary Intake

Subjects in both groups had not consumed any other protein supplements before participating in the present study. They were instructed to maintain their dietary habits during the study period; however, they were instructed to refrain from any food and fluids, except for the supplements and tap water provided in this study for >2 h before and after exercise each day. Furthermore, they were instructed to report the food consumed during the 5-day training period by answering a questionnaire prepared by a dietician. When supplement intake was excluded, total calories from daily dietary intake were 2,241 ± 71 and 2,177 ± 82 kcal and protein was 93 ± 3 and 91 ± 3 g in the CNT and Pro-CHO groups, respectively, with no significant differences between groups \((P = 0.56\) and 0.64, respectively). The total caloric intake was almost equal to 35 kcal/kg by the age-matched RDA for moderately active Japanese (14), while protein intake was 43% higher than 1.01 g/kg in the recommended daily allowance. Daily NaCl intake was estimated as ~8 g in both groups, assuming 3.7 mg NaCl/kcal of daily total calories (14).

Measurements

\( V_{\text{O}_2}\text{peak} \). \( V_{\text{O}_2}\text{peak} \) was measured with graded exercise using a cycle ergometer in an upright position at a \( T_a \) of 25.0 ± 0.1°C \((\text{mean} \pm \text{range})\) and \( \text{RH} \) of 46 ± 1%. After baseline measurements at rest for 3 min, subjects started pedaling at 60 cycles/min without loading. Exercise intensity was increased by 60 W every 3 min until 180 W and, above this intensity, by 30 W every 2 min until 240 W and then by 15 W every 2 min until exhaustion. \( V_{\text{O}_2} \) was determined every 15 s (Aeromonitor AE260; Minato, Tokyo) and \( H_R \) was recorded every minute (ECG; Life Scope 8; Nihon Kohden, Tokyo). \( V_{\text{O}_2}\text{peak} \) was determined by averaging the three largest consecutive values at the end of exercise.

PV, BP, and blood and plasma constituents. On the day of measurement, subjects reported to the laboratory at 0700 normally hydrated but without having eaten any food for at least 12 h before the measurement. To ensure that they were well hydrated, they were asked to drink 10 ml/kg water 2 h prior to the visit. After emptying their bladders, they were weighed in the nude and then, clad in shorts and shoes, entered an environmental chamber controlled to a \( T_a \) of 28.0 ± 0.1°C \((\text{mean} \pm \text{range})\) and RH of 50 ± 1%. An 18-gauge Teflon catheter was then placed in the right antecubital vein for blood sampling and dye injections. After subjects had rested quietly in a sitting position for >45 min, PV was determined by the Evans blue dye dilution method (8, 22). Briefly, after baseline blood samples were taken, the dye was injected and blood samples were taken 10 and 20 min after injection, and the absorbance (620 and 740 nm, U-1500; Hitachi, Tokyo) of a 10-min plasma sample was used to calculate PV. Blood volume (BV) was calculated from PV and hematocrit (Hct) values after correction for plasma trapped among red blood cells in the Hct tube (0.96) and for the F-cell ratio (0.91) (9), assuming that the F-cell ratio for healthy adults is not related to aerobic fitness (25). Red cell volume (RCV) was calculated from BV – PV.

An aliquot of the baseline blood sample was transferred to a heparin-treated tube and used to determine Hct (%), microcentrifuge and hemoglobin concentration ([Hb], g/dl cyanomethohemoglobin method; Sigma Chemical) 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 stored at –80°C until
the assays were performed. The plasma was used to determine total protein ([TP]p, g/dl), by the biuret method; Wako Chemical, Tokyo, Japan), albumin concentration ([Alb]a, g/dl, by the bromcresol green method; Wako Chemical), and plasma sodium concentration ([Na+]p, mmol/kgH2O, by flamephotometry; Flamephotometer 480; Corning, Medfield, MA). Plasma globulin concentration ([Glb]p) was calculated as [TP]p - [Alb]p. Total circulating plasma protein (TPcont, Albcont, and globulin (Glbcont) were calculated as products of PV and [TP]p, [Alb]p, or [Glb]p, respectively. [Na+]p is presented in millimoles per kilogram H2O after correction for [TP]p.

Thermoregulatory response test. After the PV measurement, subjects emptied their bladders, were weighed again in the nude, and then, clad in shorts and shoes, entered the environmental chamber controlled to 30.0 ± 0.1°C Ta and 50 ± 1% RH (mean ± range) at 1100. Subjects rested quietly in a semi-recumbent position in the contoured chair of the cycle ergometer for 60 min while all measurement devices were applied. The reasons for adopting the position were to have subjects’ forearms relaxed to measure skin blood flow in the left arm by strain-gauge plethysmography and to sample non-congested blood from the superficial cutaneous vein of the right arm during exercise. After resting, baseline measurements were taken for 10 min, and subjects performed cycling exercise in the semi-recumbent position at 65% of their pretraining Vo2peak for 30 min without fan cooling. Blood samples were taken 10 and 5 min before and 5, 10, 20, and 30 min after the start of exercise and used to determine Hct and [Hb] as described above. HR, BP, esophageal temperature (Tes), mean skin temperature (Tsk), chest sweat rate (SR), forearm skin blood flow (FBF), and cardiac output (CO) were measured as described below. After the test, subjects wiped off the sweat and were then weighed again in the nude.

HR and BP. During the thermoregulatory response test, HR was recorded every minute as described above and systolic (SBP) and diastolic (DBP) BP were measured every minute from the right upper arm at the heart level by inflation of the cuff with sonometric pickup recorded every minute as described above and systolic (SBP) and diastolic (DBP) BP were measured every minute from the right upper arm at the heart level by inflation of the cuff with sonometric pickup recorded every minute as described above. HR drift (HRdrift) during exercise in the thermoregulatory response test was determined by subtracting HR at 5 min of exercise from that at 30 min of exercise.

Tes and Tsk. Tes, Tsk, was monitored with a thermocouple in polyethylene tubing (PE-90). The tip of the tube was advanced to a distance of one-fourth of the subject’s standing height from the external nares. Tsk was monitored as Tsk = 0.25·Tes + 0.43·Tch + 0.32·Tth (23), where Tsk, Tch, and Tth were skin surface temperatures; the right forearm at 10 cm below the cubital line on the radial line, the right chest at 10 cm below the midclavicle, and the right anterior thigh at 15 cm above the patella on the middle line, which were measured with thermocouples, respectively. Tes and Tsk were recorded every 5 s and presented every minute on average. An increase in Tes (ΔTes) during exercise in the thermoregulatory response test was determined by subtracting Tes at baseline from that at 30 min of exercise as an index of heat storage in the body.

SR and FBF. SR was determined by capacitance plethysmography, calculated from the relative humidity and temperature (THP-B3T; Shineti, Tokyo, Japan) of the air flowing out of a 12.56 cm² capsule at the rate of 1.5 l/min on the chest 5 cm below the left midclavicle. FBF was measured by venous occlusion plethysmography with a mercury-in-Silastic tube strain gauge placed around the upper side of the subject’s 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) (29). SR was recorded every 5 s and FBF was measured twice every minute and presented every minute on average. In addition, total sweat volume during 30-min exercise was calculated from changes in body weight before and after exercise.

CO. CO was measured at the 10th and 5th min before the start of exercise and at the 5th, 10th, and 29th min after the start of exercise by the transcutaneous indocyanine green (ICG) dilution method (12). Briefly, ICG (Daiichi, Tokyo, Japan) dissolved in 0.9 ml saline (5 mg/ml) was placed in a 1-ml syringe and transferred to a filled extension tube connected to the Teflon catheter placed in the antecubital vein via a three-way stopcock. The dye was then injected with 10–15 ml saline solution by swiftly pushing the inner cylinder of a 20-ml syringe connected to the stopcock so as not to leave the dye in the extension tube. Several seconds after injection, transient changes in ICG concentration in arterial blood were monitored transcutaneously with detectors placed on the nostril, and traces from each detector were printed out by the instrument (DDG-2001; Nihon Kohden). CO was calculated after correction for the injected amount of ICG and [Hb] in blood samples taken prior to dye injection. SV was calculated from CO and HR measured at the same time. The reproducibility of this measurement, examined in another group of young subjects during graded cycle ergometer exercise, was confirmed to be 0.4–0.9 l/min of 95% confidence limit over the range of 3.3–22.8 l/min, covering the range in the present study (21).

Data Analyses

Forearm vascular conductance. Forearm vascular conductance (FVC) was calculated as FBF/MBP as a unit of milliliters per 100 milliliters per minute per 100 millimeter of Hg.

SR and FVC responses to increased Tes. The Tes threshold for increasing SR (THSR) and FVC (THFVC) and the sensitivity of the increase in SR (ΔSR/ΔTes) and FVC (ΔFVC/ΔTes) at a given increase in Tes were determined from the Tes vs. SR or FVC in each subject, as described previously (11). Briefly, the Tes vs. SR relationship in each subject was fitted with three linear regression lines determined visually. The first was determined from the first sharp increase in Tes before the rapid increase in SR, the second was determined from the rapid increase in SR, and the third was determined from measurements after the second component. The THSR was determined from the cross point of the first and second regression lines. The ΔSR/ΔTes was determined on the second component. The THSR and ΔSR/ΔTes were determined and the three values were averaged. The THFVC and ΔFVC/ΔTes were determined by the same methods. These determinations were performed by three separate investigators who were familiar with the method but blinded to the groups to which subjects belonged, and the three values were averaged.

Statistics

Values are expressed as the means ± SE for 9 subjects in each group. Two-way [1 between (group) and 1 within (training)] ANOVA for repeated measures was used to test any significant effect of training on variables in each group (Tables 1, 2, and 4). Similarly, three-way [1 between (group) and 2 within (training and time)] ANOVA for repeated measures was used to test any significant effect of training on variables at any times during the thermoregulatory response test in each group (Table 3, Figs. 2 and 3). To examine any significant differences in variables after training between groups, they were corrected for their pretraining values as covariates by ANCOVA and then used for the following analyses. Unpaired t-test was used to examine any significant differences in variables between groups before and after training, respectively, in Tables 1, 2, and 4. Two-way [1 between (group) and 1 within (time)] ANOVA was used to examine any significant differences in variables at any times during the thermoregulatory response test between groups before and after training, respectively, in Table 3, Fig. 2, A-D, and Fig. 3. When significant differences were observed by ANOVA, subsequent post hoc tests to determine significant differences in various pairwise comparisons were performed using Fisher’s least significant difference test. The unpaired t-test was used to examine any significantly different effects between groups on percent.
changes in PV, TPcont, and Albcont (Fig. 1) and on a reduction of the increase of Tes or HR during the thermoregulatory response test after training (Fig. 2, E and F). The null hypothesis was rejected when \( P < 0.05 \).

Table 1 shows the physical characteristics of subjects before and after training. After training, \( \bar{V}O_2^{\text{peak}} \) increased significantly by 3.1% in the CNT group and by 4.8% in the Pro-CHO group (both, \( P < 0.001 \)) while other variables did not. There were no significant differences between groups after training in any variables.

As in Table 2, after training, BV and PV increased in both groups (both, \( P < 0.001 \)). \([\text{Alb}]_p\) increased significantly in Pro-CHO (\( P < 0.001 \)) but did not in CNT (\( P = 0.50 \)). \([\text{Glb}]_p\) decreased significantly in both groups (CNT, \( P = 0.016 \); Pro-CHO, \( P < 0.001 \)), and \([\text{TP}]_p\) and \([\text{Na}^+]_p\) remained unchanged in both groups. TPcont and Albcont increased (both, \( P < 0.001 \)) while Glbcont increased minimally in both groups. We found that BV (\( P = 0.022 \)), PV (\( P = 0.009 \)), \([\text{Alb}]_p\), TPcont, and Albcont after training were significantly higher in Pro-CHO than CNT (all, \( P < 0.001 \)). Indeed, we confirmed that percent changes from before training (% in PV (\( P = 0.007 \)), TPcont and Albcont (both, \( P < 0.001 \)) were significantly higher in Pro-CHO than CNT as in Fig. 1.

Figure 2 shows Tes and HR responses during the thermoregulatory response test. After training, Tes decreased significantly at rest and during exercise compared with before training in both groups (\( P < 0.001 \)) except from 2 to 8 min of exercise in CNT (\( P = 0.05 \)). Similarly, HR decreased significantly at rest and during exercise compared with before training in both groups (both, \( P < 0.001 \)); however, we found that Tes and HR after training were significantly lower in Pro-CHO than CNT at rest (\( P = 0.007 \) and \( P = 0.09 \), respectively) and during exercise (\( P = 0.006 \) and \( P = 0.005 \), respectively).
presented. FVC during exercise increased after training in both groups. As summarized in Table 4, ΔSR/ΔTes increased in CNT (P = 0.052) and Pro-CHO (P < 0.001). ΔSR/ΔTes was significantly higher in Pro-CHO than CNT after training (P = 0.018). ΔFVC/ΔTes increased in Pro-CHO (P < 0.001) while it remained unchanged in CNT (P = 0.16) but with no significant difference between groups after training. Indeed, we confirmed that the increase in ΔSR/ΔTes in Pro-CHO (39.5%) was significantly greater than CNT (9.5%) (P = 0.029) while the increase in ΔFVC/ΔTes in Pro-CHO (52.9%) was marginally but not significantly greater than CNT (19.3%) (P = 0.15). THSR remained unchanged after training in both groups. In contrast, THFVC decreased significantly in Pro-CHO (P < 0.001) but remained unchanged in CNT (P = 0.16). There were no significant differences in THSR and THFVC between groups after training (P = 0.52 and 0.59, respectively).

We assessed any contribution of the increase in PV after training to the increases in SV and thermoregulatory responses on the data pooled from individual subjects in both groups and found that the increase in PV at Ex10, around the time of the rapid increases in FVC and SR, was significantly correlated with that in ΔFVC/ΔTes (r = 0.506, P = 0.032) but not with ΔSR/ΔTes (r = 0.408, P = 0.093). We did not find any significant correlations between the increases in PV and SV after training at any time of exercise (r = 0.139–0.457, P = 0.057–0.582).

Table 1. Physical characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>CNT</th>
<th>Pro-CHO</th>
<th>P values (CNT versus Pro-CHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>24.1 ± 1.3</td>
<td>23.3 ± 0.9</td>
<td>n.s.</td>
</tr>
<tr>
<td>Height, cm</td>
<td>170 ± 2</td>
<td>171 ± 2</td>
<td>n.s.</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>63.7 ± 2.0</td>
<td>63.5 ± 1.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI, kg·m⁻²</td>
<td>22.2 ± 0.9</td>
<td>21.7 ± 0.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>VO2peak, ml·min⁻¹</td>
<td>3205 ± 96</td>
<td>3056 ± 87</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are the means ± SE for 9 subjects. CNT, placebo intake group; Pro-CHO, protein and carbohydrate supplement intake group. BMI, body mass index; VO2peak, peak oxygen consumption rate. *s, P < 0.05 versus before training in each group. P values between groups before (Before) and after (After) training were determined by the unpaired t-test. For the analysis, the values after training were corrected for the pretraining values as covariates by ANCOVA; however, the values in the table are those without correction. n.s., not significant.

Table 2. Blood volumes and constituents before and after 5-day training

<table>
<thead>
<tr>
<th></th>
<th>CNT Before</th>
<th>After</th>
<th>Pro-CHO Before</th>
<th>After</th>
<th>P values (CNT versus Pro-CHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV, ml</td>
<td>4913 ± 170</td>
<td>5045 ± 167*</td>
<td>5002 ± 208</td>
<td>5249 ± 200*</td>
<td>n.s.</td>
</tr>
<tr>
<td>PV, ml</td>
<td>3004 ± 93</td>
<td>3122 ± 97*</td>
<td>2991 ± 135</td>
<td>3212 ± 132*</td>
<td>n.s.</td>
</tr>
<tr>
<td>RCV, ml</td>
<td>1910 ± 83</td>
<td>1923 ± 76</td>
<td>2010 ± 86</td>
<td>2038 ± 94</td>
<td>n.s.</td>
</tr>
<tr>
<td>[TP]g·dl⁻¹</td>
<td>6.63 ± 0.11</td>
<td>6.57 ± 0.10</td>
<td>6.70 ± 0.10</td>
<td>6.69 ± 0.08</td>
<td>n.s.</td>
</tr>
<tr>
<td>[Alb]g·dl⁻¹</td>
<td>4.20 ± 0.06</td>
<td>4.20 ± 0.05</td>
<td>4.20 ± 0.04</td>
<td>4.27 ± 0.05*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>[Glb]g·dl⁻¹</td>
<td>2.43 ± 0.06</td>
<td>2.37 ± 0.05*</td>
<td>2.51 ± 0.09</td>
<td>2.41 ± 0.07*</td>
<td>n.s.</td>
</tr>
<tr>
<td>[Na⁺]mmol·kgH₂O⁻¹</td>
<td>150 ± 0</td>
<td>150 ± 0</td>
<td>149 ± 1</td>
<td>151 ± 1</td>
<td>n.s.</td>
</tr>
<tr>
<td>TPcont, g</td>
<td>199 ± 3</td>
<td>205 ± 3*</td>
<td>201 ± 10</td>
<td>215 ± 10*</td>
<td>n.s.</td>
</tr>
<tr>
<td>Albcont, g</td>
<td>126 ± 4</td>
<td>131 ± 4*</td>
<td>126 ± 6</td>
<td>137 ± 6*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glbcont, g</td>
<td>73 ± 3</td>
<td>74 ± 3*</td>
<td>75 ± 5</td>
<td>78 ± 4*</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are the means ± SE for 9 subjects. CNT, placebo intake group; Pro-CHO, protein and carbohydrate supplement intake group. BV, blood volume; PV, plasma volume; RCV, red cell volume; [TP], [Alb], [Glb], [Na⁺], plasma total protein, albumin, globulin, and sodium concentration, respectively. *s, P < 0.05 versus before training in each group. P values between groups before (Before) and after (After) training were determined by the unpaired t-test. For the analysis, the values after training were corrected for the pretraining values as covariates by ANCOVA; however, the values in the table are those without correction. n.s., not significant.
PV and Albcont after 5-day aerobic training were enhanced and (Pro-CHO than CNT was almost identical to the greater in-warm environment. In the present study, we reconfirmed this after 5-day aerobic training in a warm environment. As Senay et al. (27) suggested, since albumin has a low plasma protein had been regarded as only sustaining PV (26). Greater increases in PV and Albcont in Pro-CHO

As in Table 2 and Fig. 1, the increases in PV, TPcont, and Albcont after training were twofold higher in Pro-CHO than in CNT with significance, whereas the increase in Glbcont after training was minimal in both groups with no significant difference between groups. In our previous study (20), we suggested that protein and CHO intake immediately after a bout of intense exercise enhanced Albcont, starting 1 h after the cessation of exercise and lasting for the next 22 h. In the present study, we reconfirmed this after 5-day aerobic training in a warm environment.

As in Fig. 1, we found that the greater increase in PV in Pro-CHO than CNT was almost identical to the greater increase in Albcont but with no significant difference in the increase of Glbcont between groups (Table 2). Senay et al. (27) first suggested the role of albumin in heat acclimation-induced PV expansion, although previously the oncotic qualities of plasma protein had been regarded as only sustaining PV (26). As Senay et al. (27) suggested, since albumin has a low

<table>
<thead>
<tr>
<th>Table 3. Cardiovascular and body temperature responses during exercise in a warm environment before and after 5-day training</th>
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<table>
<thead>
<tr>
<th>Subject</th>
<th>PV (ml)</th>
<th>TPcont (ml)</th>
<th>Albcont (g·l−1)</th>
<th>Glbcont (g·l−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>CNT</td>
<td>Pro-CHO</td>
<td>CNT</td>
<td>Pro-CHO</td>
<td>CNT</td>
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<td>---</td>
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<td>---</td>
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</tr>
<tr>
<td>Rest</td>
<td>123 ± 7</td>
<td>120 ± 7</td>
<td>117 ± 6</td>
<td>117 ± 6</td>
</tr>
<tr>
<td>Exercise</td>
<td>139 ± 9</td>
<td>136 ± 9</td>
<td>123 ± 8</td>
<td>123 ± 8</td>
</tr>
<tr>
<td>5 min</td>
<td>136 ± 7</td>
<td>133 ± 7</td>
<td>122 ± 6</td>
<td>122 ± 6</td>
</tr>
<tr>
<td>10 min</td>
<td>135 ± 8</td>
<td>132 ± 8</td>
<td>121 ± 7</td>
<td>121 ± 7</td>
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<tr>
<td>20 min</td>
<td>134 ± 9</td>
<td>131 ± 9</td>
<td>120 ± 8</td>
<td>120 ± 8</td>
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<tr>
<td>30 min</td>
<td>133 ± 10</td>
<td>130 ± 10</td>
<td>119 ± 9</td>
<td>119 ± 9</td>
</tr>
</tbody>
</table>

Values are the means ± SE for 9 subjects. CNT, placebo intake group; Pro-CHO, protein and carbohydrate supplement intake group. MBP, mean blood pressure; Tsk, mean skin temperature; SR, chest sweat rate; FVC, forearm vascular conductance. *P < 0.05 versus before training in each group. p values between groups before (Before) and after (After) training were determined by two-way (group) and 1 within (time) ANOVA; however, the values in the table are those without correction. n.s., not significant.

**DISCUSSION**

The major findings in this study are that 1) the increases in PV and Albcont after 5-day aerobic training were enhanced when a protein and CHO supplement was given to young men immediately after daily exercise, 2) which was accompanied by reduced heat strain with enhanced sensitivity of thermoregulatory responses, and 3) also by attenuated cardiovascular strain, compared with when a placebo supplement was given.
molecular mass and is abundant in plasma, an increase in Alb_concentration and therefore temporarily increased $[\text{Alb}]_p$, moved fluid from the extra- to intravascular space according to the effective oncotic pressure gradient, and thereby induced PV expansion (2, 7). Thus 80–90% of the increase in PV could be accounted for by the increase in Alb_concentration since 1 g of albumin is known to contribute 18 ml plasma water retention (26).

Another possible mechanism for the greater increase in PV in Pro-CHO than CNT might be the enhanced actions of Na$^+$ retention hormones (2), which are reportedly secreted during strenuous exercise, accelerating reabsorption of Na$^+$ and water in the renal tubules and increasing extracellular fluid space, causing PV expansion. However, this is not plausible because experimental conditions other than supplementation (exercise regimen, dehydration level, environmental condition, and food intake) were strictly controlled between the two groups during the training period. Also, we confirmed in young subjects in the previous study (20) that the plasma aldosterone level during recovery from intense exercise was not influenced by protein and CHO supplementation immediately after exercise.

Greater increase in thermoregulatory capacity in Pro-CHO

As in Fig. 2, the increases in $T_{es}$ and HR during exercise were more attenuated after training in Pro-CHO than CNT. In addition, as in Fig. 4 and Table 4, we confirmed that the increase in $\Delta SR/\Delta T_{es}$ was twofold greater in Pro-CHO than CNT. Moreover, we confirmed that $\Delta FVC/\Delta T_{es}$ increased and $TH_{FVC}$ decreased in Pro-CHO, but remained unchanged in CNT.

As for the mechanisms for the greater improvement in thermoregulatory responses, a greater increase in PV might be involved. Many studies have suggested that an acute increase in venous return to the heart enhances the sensitivity of skin vasodilation in response to increased $T_{es}$ during exercise. Fortney et al. (5) examined the effects of acute BV expansion by $\sim 600$ ml after blood transfusion 1 h before exercise on SV and FBF responses to increased $T_{es}$ during exercise in a warm environment and suggested that the BV expansion increased SV by 20 ml/beat and FBF response by 63% compared with before transfusion. Lately, Nose et al. (19) examined the effects of intravenous saline infusion on the skin blood flow response during exercise in a warm environment and suggested that an $\sim 150$ ml increase in PV enhanced skin blood flow by 35% at 38.1°C $T_{es}$ compared with no infusion. Similarly, head-out water immersion (18) and continuous negative pressure breathing (16) during exercise, known to accelerate venous return to the heart and increase SV by 5–10% compared with that in the control (1), were suggested to enhance the sensitivity of skin vasodilation by a similar degree. Since this increase in SV was reportedly attained by $\sim 100$ ml PV expansion (24), these results suggest that even an acute and small increase in PV markedly enhances the skin blood flow response to increased $T_{es}$ during exercise.

On the other hand, few studies have supported the idea that PV expansion after aerobic training significantly contributes to the enhanced sensitivity of the cutaneous vasodilation response, although it always occurs simultaneously after training. Indeed, Takeno et al. (28) examined the effects of PV expansion on the sensitivity of the skin vasodilatory response to increased $T_{es}$ in a cool and warm environment. They found that the increase in sensitivity was less in a cool than warm environment despite the similar increase in PV. These results suggest that improvement of the thermoregulatory response after aerobic training is primarily caused by neural adaptation of the thermoregulatory center in the hypothalamus to repeated

Table 4. Thermoregulatory responses during exercise in a warm environment before and after 5-day training

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>Before</th>
<th>After</th>
<th>$P$ values (CNT versus Pro-CHO)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Before</td>
</tr>
<tr>
<td>$T_{SR}$, °C</td>
<td>36.9 ± 0.1</td>
<td>36.9 ± 0.1</td>
<td>37.1 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>$T_{Hve}$, °C</td>
<td>37.0 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>37.1 ± 0.1</td>
<td>37.0 ± 0.1</td>
<td>n.s.</td>
</tr>
<tr>
<td>$\Delta SR/\Delta T_{es}$, mg·cm$^{-2}$·min$^{-1}$·°C$^{-1}$</td>
<td>1.99 ± 0.26</td>
<td>2.18 ± 0.26</td>
<td>1.62 ± 0.23</td>
<td>2.26 ± 0.34*</td>
<td>n.s.</td>
</tr>
<tr>
<td>$\Delta FVC/\Delta T_{es}$, units·°C$^{-1}$</td>
<td>32.1 ± 5.4</td>
<td>38.3 ± 7.7</td>
<td>32.3 ± 4.8</td>
<td>49.4 ± 9.5*</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Values are the means ± SE for 9 subjects. CNT, placebo intake group; Pro-CHO, protein and carbohydrate supplement intake group. $T_{SR}$ and $T_{Hve}$, esophageal temperature ($T_{es}$) threshold for sweating and cutaneous vasodilation, respectively, $\Delta SR/\Delta T_{es}$, and $\Delta FVC/\Delta T_{es}$, sensitivity of the increase in chest sweat rate and forearm vascular conductance, respectively. *' s, $P < 0.05$ versus before training in each group. $P$ values between groups before (Before) and after (After) training were determined by unpaired $t$-test. For the test, the values after training were corrected for the pretraining values as covariates by ANCOVA; however, the values in the table are those without correction. n.s., not significant.
heat exposure during training (15) and that increased PV is not a cause of improvement but a result of adaptation.

However, in the present study, subjects in both groups underwent the same protocol of aerobic training in the same environment, strictly controlled in an artificial climate chamber. Experimentally, there were no significant differences in sweat loss during training, suggesting similar heat exposure during training between groups. As a result, the only difference between groups was greater PV expansion in Pro-CHO than CNT. In addition, we found that greater PV expansion by ~100 ml in Pro-CHO than CNT evoked greater increases in SV by ~5 ml/beat (~4%) (Fig. 3) and ΔFVC/ΔTes by ~50% (Table 4) on average, consistent with the results of previous studies examining the effects of acute increases in PV or SV on cutaneous vasodilation during exercise in a warm environment (16, 18, 19). Indeed, we found significant correlations between increases in PV and ΔFVC/ΔTes after training when they were pooled from individual subjects in both groups; however, we failed to find any significant correlations between changes in PV and ΔSR/ΔTes or SV after training, which was probably due to interindividual variances of their responses.

In the present study, we found that the greater increase in ΔFVC/ΔTes for Pro-CHO was accompanied by a greater increase in ΔSR/ΔTes, by a similar degree (Table 4), suggesting that PV expansion was also closely associated with the enhanced sweating response. On the other hand, Fortney et al. (6) suggested that the sweating response to increased Tes during exercise in a warm environment was not increased by an 8% acute increase in BV induced by the infusion of 5% human serum albumin in isotonic saline solution prior to exercise, suggesting that the sweating response was not so sensitive to BV expansion as the FVC response. This discrepancy might be explained by repeated exposure to heat stress during aerobic training in a warm environment. Convertino et al. (3) assessed the mechanisms of aerobic training-induced hypervolemia and reported that thermal as well as exercise stress during training significantly contributed to PV expansion and thermoregulatory response enhancement. Recently, Okazaki et al. (22) examined the effects of BV change on thermoregulatory responses during exercise in older subjects who underwent sedentary control, resistance training, and aerobic training interventions for 18 wk, suggesting that the changes in ΔSR/ΔTes as well as ΔFVC/ΔTes after the interventions were significantly correlated with the change in BV when the values from all subjects were pooled. More recently, Okazaki et al. (21) examined the effects of protein and CHO supplementation immediately after daily exercise during 8-wk aerobic training in older subjects and suggested that ΔSR/ΔTes as well as ΔFVC/ΔTes increased with increased BV in the supplement group while not in the placebo group. Taken together, repeated thermal and exercise stimulations of central and/or peripheral mechanisms during training enhanced the sensitivity of the sweating response to a given increase of PV in younger and older subjects.

Comparison with Older Subjects

We found in the present study that a mixture of protein and carbohydrate supplement intake during aerobic training enhanced the improvement of the thermoregulatory response to heat stress during exercise in young subjects who had higher PV, Albcont, aerobic, and thermoregulatory capacities than older subjects (21), and moreover, this occurred after a shorter training period. Regarding the gain of improved thermoregulatory responses at a given increase in PV or BV, Okazaki et al. (22) suggested in older subjects that ΔSR/ΔTes (mg·min⁻¹·m⁻²·°C⁻¹) and ΔFVC/ΔTes (units/°C) increased by 0.1 (25%) and 4.0 (36%), respectively, per 1 ml/kg increase in BV after 18-wk sedentary, resistance, or aerobic training interventions. Similarly, Okazaki et al. (21) suggested in older subjects that ΔSR/ΔTes and ΔFVC/ΔTes increased by 0.1 (25%) and 1.6 (36%) per 1 ml/kg increase in BV, respectively, after 8-wk aerobic training with protein and CHO supplementation after daily aerobic exercise. On the other hand, in the young subjects in the present study, ΔSR/ΔTes and ΔFVC/ΔTes increased by 0.2 (39%) and 4.9 (53%) with Pro-CHO. These results suggest that the relative effects of a given increase in BV or PV after training on thermoregulatory responses appeared to be greater in young than older subjects when the effects of other experimental conditions (exercise intensity, training period, and environmental conditions) are controlled.

Limitations

In the present study, we found a significant increase in Albcont in CNT, although much less than Pro-CHO after 5-day training, different from the results of the placebo supplementation group in young subjects in the previous study, suggesting a minimal increase of Albcont 23 h after a bout of intense exercise (20). Other mechanisms, such as the redistribution of albumin from the extra- to intravascular space (13) and the reduced transcapillary escape rate of albumin (10) after exercise, should be considered in addition to the different exercise regimen (continuous exercise at 70% VO2peak for 30 min/day at 30°C and ~50% RH, repeated for 5 consecutive days) from the previous study (21). Alternatively, average protein intake was estimated as 1.45 g·kg⁻¹·day⁻¹ during the training period in the present study, much higher than the 1.01 g·kg⁻¹·day⁻¹ in young subjects in the previous study (21), which might have increased Albcont in CNT, although the timing of protein intake after daily exercise was not identified.

In conclusion, these results suggest that the increases in PV and Albcont were enhanced by a mixture of protein and CHO supplement intake during 5-day aerobic training in young men, which was accompanied by reduced heat strain with enhanced sensitivity of thermoregulatory responses and also by attenuated cardiovascular strain. These results may provide a new training regimen to facilitate cardiovascular and thermoregulatory adaptations in young people, although the optimal composition of the supplement, the dose response of protein, and the duration of effectiveness by the supplement remains to be addressed in the future.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
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