J Physiol 587.23 (2009) pp 5585–5590

The Journal of Physiology

SYMPOSIUM REVIEW

Protein and carbohydrate supplementation increases aerobic and thermoregulatory capacities

Kazunobu Okazaki, Masaki Goto and Hiroshi Nose

Department of Sports Medical Sciences, Shinshu University Graduate School of Medicine, Matsumoto 390-8621, Japan

The incidence of heat illness and heat stroke is greater in older than younger people. In this context, exercise training regimens to increase heat tolerance in older people may provide protection against heat illness. Acute increases in plasma volume (PV) improve thermoregulation during exercise in young subjects, but there is some evidence that changes in PV in response to acute exercise are blunted in older humans. We recently demonstrated that protein–carbohydrate (Pro-CHO) supplementation immediately after a bout of exercise increased PV and plasma albumin content (Alb_{cont}) after 23 h in both young and older subjects. We also examined whether Pro-CHO supplementation during aerobic training enhanced thermoregulation by increasing PV and Alb_{cont} in older subjects. Older men aged ∼68 years exercised at moderate intensity, 60 min day^{-1}, 3 days week^{-1}, for 8 weeks, at ∼19°C, and took either placebo (CNT; 0.5 kcal, 0 g protein kg^{-1}) or Pro-CHO supplement (Pro-CHO; 3.2 kcal, 0.18 g protein kg^{-1}) immediately after exercise. After training, we found during exercise at 30°C that increases in oesophageal temperature (T_{es}) were attenuated more in Pro-CHO than CNT and associated with enhanced cutaneous vasodilatation and sweating. We also confirmed similar results in young subjects after 5 days of training. These results demonstrate that post-exercise protein and CHO consumption enhance thermoregulatory adaptations especially in older subjects and provide insight into potential strategies to improve cardiovascular and thermoregulatory adaptations to exercise in both older and younger subjects.

(Received 20 August 2009; accepted after revision 11 September 2009; first published online 14 September 2009)
Corresponding author K. Okazaki: Research Centre for Urban Health and Sports, Osaka City University, and Department of Environmental Physiology for Exercise, Osaka City University Graduate School of Medicine, 3-3-138 Sugimoto, Sumiyoshi, Osaka 558-8585, Japan. Email: okazaki@sports.osaka-cu.ac.jp

Introduction

The incidences of heat illness and heat stroke during mid-summer have rapidly increased these two decades as ambient temperatures (T_a) increased globally (Nakai *et al.* 2007). This tendency was more prominent in the elderly than young adults (Klinenberg, 2003; Nakai *et al.* 2007) who are less tolerant to heat stress (Kenney, 1997). Nakai *et al.* (2007) suggested that 1935 older people, aged 70–90 years, died from heat stroke between 1968 and 2005 in Japan, 82% higher than 1065 in younger people, 45–65 years, based on the databases accumulated from the reports by the news papers during the period (Fig. 1). To prevent this, aerobic training is recommended to increase thermoregulatory capacity in both older people (Thomas *et al.* 1999; Kenney & Munce, 2003) and young adults (Roberts *et al.* 1977; Ichinose *et al.* 2005). However, the beneficial effects of training on thermoregulation are generally attenuated in older adults compared with those in young subjects (Ho *et al.* 1997; Okazaki *et al.* 2002) although the precise mechanisms remain unknown.

Indeed, we confirmed that cutaneous blood flow and sweat rate responses to exercise remained unchanged on average after 18 weeks of aerobic training in older subjects (Okazaki *et al.* 2002). However, in this study, we found that the responses were enhanced in a subset of older subjects with increased plasma volume (PV) while reduced in subjects with a blunted PV response to training, suggesting that the attenuated improvement of thermoregulation after aerobic training in older people was due to attenuated PV expansion.

This review was presented at *The Journal of Physiology* Symposium on *Physiological regulation linked with physical activity and health*, which took place at the 36th International Congress of Physiological Sciences in Kyoto, Japan on 31 July 2009. It was commissioned by the Editorial Board and reflects the views of the authors.

DOI: 10.1113/jphysiol.2009.180562

Several studies on the effects of acute PV expansion on thermoregulation in young subjects have suggested that an acute increase in cardiac filling pressure by saline infusion (Nose *et al.* 1990), head out water immersion (Nielsen *et al.* 1984), or continuous negative pressure breathing (Nagashima *et al.* 1998) enhanced cutaneous vasodilatation due to an increased cardiac stroke volume (SV) during exercise in a warm environment. These results suggest that stretching of cardiac wall by PV expansion enhances the sensitivity of thermoregulation via baroreflex mechanisms that facilitate high levels of skin blood flow. On the other hand, there have been few studies, regardless of subject age, demonstrating that PV expansion after aerobic training significantly contributes to the enhanced thermoregulatory sensitivity since central nervous system adaptations to heat stress also occur (Nadel *et al.* 1974) making it difficult to distinguish the effects of PV expansion from central mechanisms.

In this paper, we briefly review our data showing that post-exercise protein and carbohydrate (CHO) supplementation during aerobic training enhanced PV expansion and accelerated cardiovascular and thermoregulatory adaptation in older subjects as well as in young subjects. This approach may be useful to increase heat tolerance especially in older people with lower thermoregulatory capacity, and our data reinforce the idea that post-exercise nutrition is critical especially for older humans.

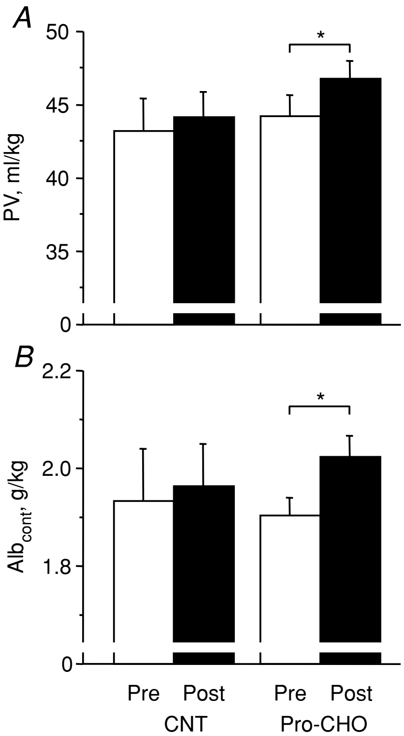

Figure 2. Plasma volume (PV) (*A*) and albumin content (Alb_{cont}) (*B*) before (Pre) and after (Post) 8 weeks of aerobic training in older men

CNT, placebo intake group; Pro-CHO, protein and carbohydrate supplement intake group. Means and S.E.M. bars are presented for 7 subjects. *$P < 0.05$. (Modified from Okazaki *et al.* 2009*b*; used with permission from the American Physiological Society.)

Post-exercise protein and CHO supplementation enhances PV expansion

The exercise-induced PV expansion in young subjects has been suggested to be oncotically mediated and so to rely on a rapid increase in plasma albumin content (Alb_{cont}), resulting in drawing fluids into the vascular space so that plasma albumin concentration remains constant (Convertino *et al.* 1980; Gillen *et al.* 1991). On the other hand, several previous studies suggested that an increase in Alb_{cont} after aerobic training was diminished in older subjects with an attenuated increase in PV (Zappe *et al.* 1996; Okazaki *et al.* 2002). Since one of the mechanisms of increased Alb_{cont} after aerobic training is likely to be enhanced response of hepatic albumin synthesis to exercise in young subjects (Yang *et al.* 1998; Nagashima *et al.* 2000), the lower increase in Alb_{cont} after training in older subjects could be caused in part by their blunted

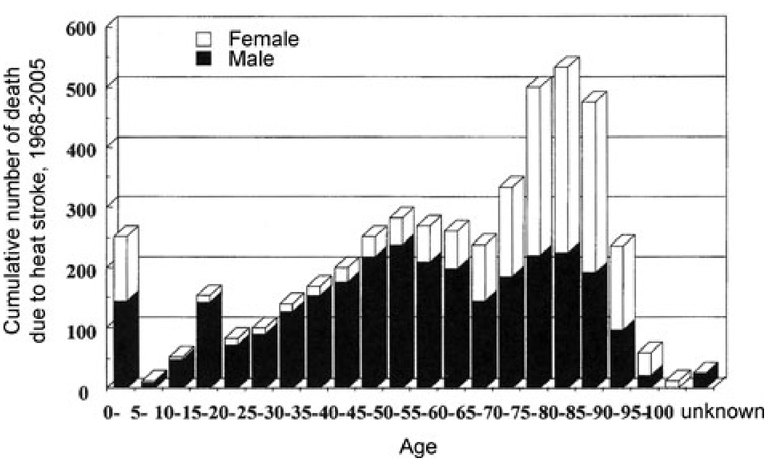

Figure 1. The cumulative number of deaths due to heatstroke from 1968 to 2005 in Japan

Total number of deaths was 5433 and each column indicates the number in each age bin of 5 years. (Modified from Nakai *et al.* 2007; used with permission.)

response of albumin synthesis to exercise (Gersovitz *et al.* 1980; Sheffield-Moore *et al.* 2004) due to a reduced gene expression rate with ageing (Horbach *et al.* 1984). However, it is also plausible that this is caused by protein intake insufficient for albumin synthesis in older people since they are likely to be habituated to low caloric and protein diets due to minimal daily physical activity (Ministry of Health, 1999). These mechanisms may lead to a reduction in substrate availability for protein synthesis after strenuous exercise in older subjects.

Therefore, we examined if increased PV and Alb_{cont} for 23 h after exercise was attenuated in older subjects compared with those in young adult subjects, and if the attenuation was abated by supplementation of protein and CHO immediately after exercise (Okazaki *et al.* 2009*a*). To do this, moderately active older aged ∼68 years and young men aged ∼21 years performed two trials: placebo (CNT; 0.5 kcal, 0 g protein per kg body weight) or protein and CHO mixture (Pro-CHO; 3.2 kcal, 0.18 g protein per kg body weight) supplementations immediately after a high-intensity interval exercise for 72 min; 8 sets of 4 min at 70–80% peak oxygen consumption rate ($\dot{V}_{O_2peak}$) followed by 5 min at 20% $\dot{V}_{O_2peak}$. PV and Alb_{cont} were measured before exercise, at the end of exercise, every hour from the first 5 hours and at the 23rd hour after exercise. The recoveries of PV and Alb_{cont} were generally attenuated in older than young subjects in the CNT trial. However, in the Pro-CHO trial, Alb_{cont} recovered more than in the CNT trial in older subjects, and was similar to the recovery in young subjects, and accompanied by a greater increase in PV.

As for the mechanisms, our data suggest that Pro-CHO supplementation enhanced the post-exercise plasma albumin synthetic rate (Yang *et al.* 1998; Nagashima *et al.* 2000; Sheffield-Moore *et al.* 2004) to increase Alb_{cont}, which in turn enhanced the effective colloid osmotic pressure gradient between intra- and extravascular spaces and thereby induced PV expansion (Convertino *et al.* 1980; Gillen *et al.* 1991).

The effects of PV expansion by protein and CHO supplementation on cardiovascular and thermoregulatory capacities after training

Next, we examined the effects of the protein and CHO supplementation during aerobic training on PV and cardiovascular and thermoregulatory responses to exercise

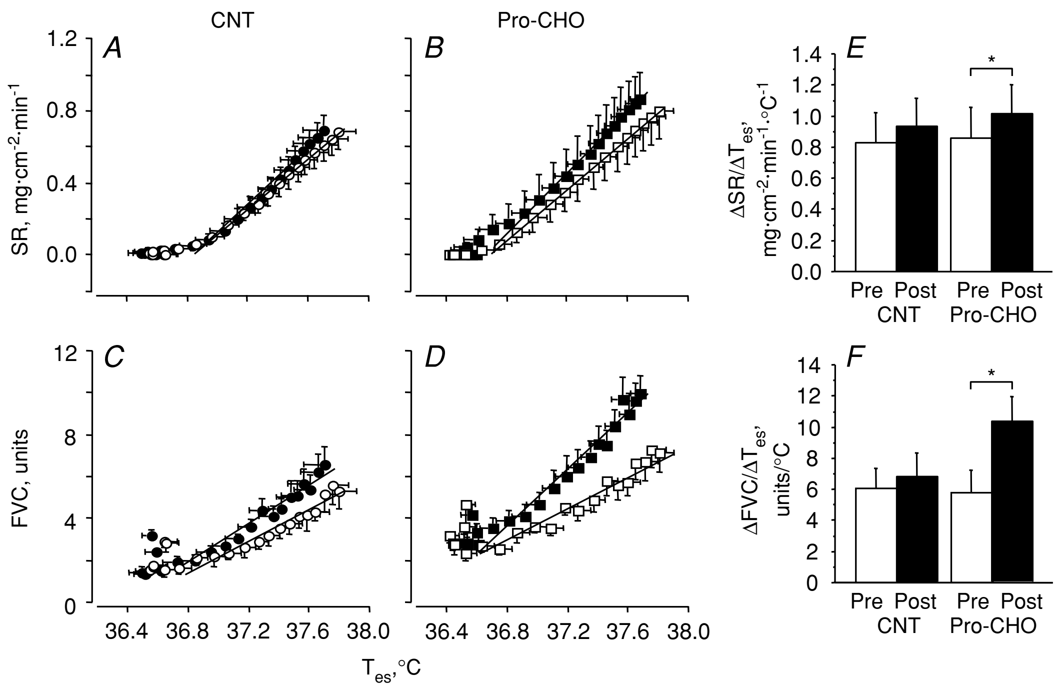

Figure 3 Thermoregulatory responses to exercise before and after 8 weeks of aerobic training in older men

A–D, chest sweat rate (SR) (*A* and *B*) and forearm skin vascular conductance (FVC) (*C* and *D*) responses to increased oesophageal temperature (T_{es}) during exercise in a warm environment (30°C ambient temperature, 50% relative humidity) before (open symbols) and after (filled symbols) training. *E* and *F* represent sensitivity of an increase in SR ($\Delta SR/\Delta T_{es}$) and FVC ($\Delta FVC/\Delta T_{es}$) at a given increase in T_{es} determined for each subject before (Pre) and after (Post) training. Exercise intensity was 60% of pre-training peak oxygen consumption rate. CNT, placebo intake group; Pro-CHO, protein and carbohydrate supplement intake group. Means and S.E.M. bars are presented for 7 subjects. *$P < 0.05$. (From Okazaki *et al.* 2009*b*; used with permission from the American Physiological Society.)

in older subjects (Okazaki *et al.* 2009*b*). Healthy older men aged ∼68 years were divided into two groups: placebo (CNT; 0.5 kcal and 0 g protein kg^{-1}) and protein and CHO mixture (Pro-CHO; 3.2 kcal and 0.18 g protein kg^{-1}) supplementations. Subjects in both groups performed an exercise training for 8 weeks; cycling exercise, 60–75% $\dot{V}_{O_2peak}$, 60 min day^{-1}, 3 days week^{-1} at T_a of ∼19°C and ∼43% relative humidity (RH) and took the respective supplement immediately after each day of exercise. After training, as shown in Fig. 2, PV and Alb_{cont} remained unchanged in the CNT group, confirming previous results from us after 18 weeks of aerobic training in older men (Okazaki *et al.* 2002) and others (Zappe *et al.* 1996; Stachenfeld *et al.* 1998; Takamata *et al.* 1999). On the other hand, in the Pro-CHO group, both PV and Alb_{cont} increased significantly by 6%.

Regarding thermoregulatory response to exercise, as shown in Fig. 3, after training, the sensitivity of chest sweat rate ($\Delta SR/\Delta T_{es}$) and forearm skin vascular conductance ($\Delta FVC/\Delta T_{es}$) in response to increased oesophageal temperature (T_{es}) during exercise at 60% pre-training $\dot{V}_{O_2peak}$, at 30°C of T_a and 50% of RH remained unchanged in the CNT group, confirming the results in our previous study (Okazaki *et al.* 2002). On the other hand, in the Pro-CHO group, the responses were significantly enhanced by 18% and 80%, respectively. Moreover, as shown in Fig. 4, after training, SV during exercise remained unchanged in the CNT group while it increased significantly by ∼10% in the Pro-CHO group. Furthermore, although an increase in heart rate (HR) during prolonged exercise in the heat was reduced in both groups, the reduction was more in the Pro-CHO group than in the CNT group. In addition, thermal strain calculated as the increase in T_{es} from before to 20 min exercise was significantly attenuated in the Pro-CHO group but did not change in the CNT group. Thus, in older men, although cardiovascular and thermoregulatory adaptation after aerobic training is generally attenuated compared to young subjects, post-exercise protein and CHO supplementation during aerobic training appears to normalize these responses by increasing PV.

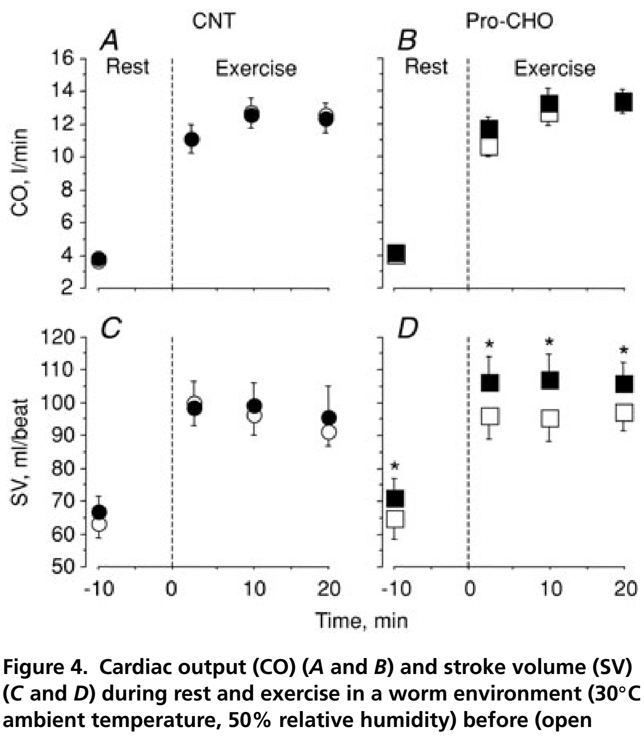

Figure 4. Cardiac output (CO) (*A* and *B*) and stroke volume (SV) (*C* and *D*) during rest and exercise in a worm environment (30°C ambient temperature, 50% relative humidity) before (open symbols) and after (filled symbols) 8 weeks of aerobic training in older men

Exercise intensity was 60% of pre-training peak oxygen consumption rate. CNT, placebo intake group; Pro-CHO, protein and carbohydrate supplement intake group. Means and S.E.M. bars are presented for 7 subjects. *$P < 0.05$ compared with before training. (From Okazaki *et al.* 2009*b*; used with permission from the American Physiological Society.)

We recently examined whether this would occur also in young men using a similar experimental protocol as in older subjects although the aerobic training period was 5 days (Goto *et al.* 2007). Young men aged ∼23 years were divided into two groups: placebo (CNT; 0.9 kcal and 0 g protein kg^{-1}), and protein and CHO mixture (Pro-CHO; 3.6 kcal and 0.36 g protein kg^{-1}) supplementations. Subjects in both groups performed 5 consecutive days of exercise training (cycling exercise, ∼70% $\dot{V}_{O_2peak}$, 30 min day^{-1}, at 30°C of T_a and 50% of RH) and took the respective supplement immediately after each day of exercise. After training, we found that PV and Alb_{cont} in the Pro-CHO group increased by ∼8% and ∼10%, respectively, which were significantly higher than ∼4% in the CNT group. During exercise, we found that the increases in HR and T_{es} during exercise were attenuated after training in both groups but significantly more in the Pro-CHO group than in the CNT group. Moreover, $\Delta SR/\Delta T_{es}$ and $\Delta FVC/\Delta T_{es}$ in the Pro-CHO group increased by 44% and 56%, respectively, after training, much higher than the 10% and 19% in the CNT group. In addition, after training, SV increased in both groups but more prominently in the Pro-CHO group than in the CNT group. Thus, thermoregulatory and aerobic adaptations to aerobic training were also enhanced by Pro-CHO supplementation in younger subjects, similarly to in older subjects.

Regarding the mechanisms for improved cardiovascular and thermoregulatory responses by the protein and CHO supplementation in both age groups, the increased PV after training with the supplementation would enhance venous return to the heart to increase cardiac filling pressure. This would further enhance SV and thermoregulatory responses by stretching the cardiopulmonary mechanoreceptors as suggested in young subjects with an acute increase in PV (Nielsen *et al.* 1984; Nose *et al.* 1990; Nagashima *et al.* 1998), and thereby reduce cardiovascular and thermal strain during exercise by permitting greater increases in skin blood flow. Thus, our results clearly demonstrate that cardiovascular and thermoregulatory capacities increase with PV expansion after training in addition to neural adaptations seen in the thermoregulatory centre in the hypothalamus to repeated heat exposure during training (Nadel *et al.* 1974).

In conclusion, aerobic training with post-exercise protein and CHO supplementation increases PV and therefore cardiovascular and thermoregulatory capacities in both elderly and young. These results provide new insight into a regimen of training and dietary manipulations to improve cardiovascular and thermoregulatory capacity more than exercise alone and prevent heat disorders in older people. They also highlight the benefits of optimizing post-exercise nutrition in both younger and older subjects.

References

Convertino VA, Brock PJ, Keil LC, Bernauer EM & Greenleaf JE (1980). Exercise training-induced hypervolemia: role of plasma albumin, rennin, and vasopressin. *J Appl Physiol* **48**, 665–669.

Gersovitz M, Munro HN, Udall J & Young VR (1980). Albumin synthesis in young and elderly subjects using a new stable isotope methodology: response to level of protein intake. *Metabolism* **29**, 1075–1086.

Gillen CM, Lee R, Mack GW, Tomaselli CM, Nishiyasu T & Nadel ER (1991). Plasma volume expansion in humans after a single intense exercise protocol. *J Appl Physiol* **71**, 1914–1920.

Goto M, Kamijo Y, Okazaki K, Masuki S, Miyagawa K & Nose H (2007). Protein and carbohydrate supplementation during 5-day aerobic training enhanced improvement of thermoregulation in young men. *FASEB J* **21**, A1296.

Ho CW, Beard JL, Farrell PA, Minson CT & Kenney WL (1997). Age, fitness, and regional blood flow during exercise in the heat. *J Appl Physiol* **82**, 1126–1135.

Horbach GJ, Princen HM, Van Der Kroef M, Van Bezooijen CF & Yap SH (1984). Changes in the sequence content of albumin mRNA and in its translational activity in the rat liver with age. *Biochim Biophys Acta* **783**, 60–66.

Ichinose T, Okazaki K, Masuki S, Mitono H, Chen M, Endoh H & Nose H (2005). Ten-day endurance training attenuates the hyperosmotic suppression of cutaneous vasodilation during exercise but not sweating. *J Appl Physiol* **99**, 237–243.

Kenney WL (1997). Thermoregulation at rest and during exercise in healthy older adults. *Exerc Sport Sci Rev* **25**, 41–76.

Kenney WL & Munce TA (2003). Invited review: aging and human temperature regulation. *J Appl Physiol* **95**, 2598–2603.

Klinenberg E (2003). Review of heat wave: social autopsy of disaster in Chicago. *N Engl J Med* **348**, 666–667.

Ministry of Health Labour, and Welfare, Japan, Study Circle for Health and Nutrition Information (1999). *Recommended Dietary Allowances for Japanese, 6th Revision. Dietary Reference Intakes [in Japanese]*. Daiichi-Shuppan Co. Ltd., Tokyo.

Nadel ER, Pandolf KB, Roberts MF & Stolwijk JA (1974). Mechanisms of thermal acclimation to exercise and heat. *J Appl Physiol* **37**, 515–520.

Nagashima K, Cline GW, Mack GW, Shulman GI & Nadel ER (2000). Intense exercise stimulates albumin synthesis in the upright posture. *J Appl Physiol* **88**, 41–46.

Nagashima K, Nose H, Takamata A & Morimoto T (1998). Effect of continuous negative-pressure breathing on skin blood flow during exercise in a hot environment. *J Appl Physiol* **84**, 1845–1851.

Nakai S, Shin-ya H, Yoshida T, Yorimoto A, Inoue Y & Morimoto T (2007). Proposal of new guidelines for prevention of heat disorders during sports and daily activities based on age, clothing and heat acclimatization. *Jpn J Fitness Sports Med* **56**, 437–444.

Nielsen B, Rowell LB & Bonde-Petersen F (1984). Cardiovascular responses to heat stress and blood volume displacements during exercise in man. *Eur J Appl Physiol Occup Physiol* **52**, 370–374.

Nose H, Mack GW, Shi XR, Morimoto K & Nadel ER (1990). Effect of saline infusion during exercise on thermal and circulatory regulations. *J Appl Physiol* **69**, 609–616.

Okazaki K, Hayase H, Ichinose T, Mitono H, Doi T & Nose H (2009*a*). Protein and carbohydrate supplementation after exercise increases plasma volume and albumin content in older and young men. *J Appl Physiol* **107**, 770–779.

Okazaki K, Ichinose T, Mitono H, Chen M, Masuki S, Endoh H, Hayase H, Doi T & Nose H (2009*b*). Impact of protein and carbohydrate supplementation on plasma volume expansion and thermoregulatory adaptation by aerobic training in older men. *J Appl Physiol* **107**, 725–733.

Okazaki K, Kamijo Y, Takeno Y, Okumoto T, Masuki S & Nose H (2002). Effects of exercise training on thermoregulatory responses and blood volume in older men. *J Appl Physiol* **93**, 1630–1637.

Roberts MF, Wenger CB, Stolwijk JA & Nadel ER (1977). Skin blood flow and sweating changes following exercise training and heat acclimation. *J Appl Physiol* **43**, 133–137.

Sheffield-Moore M, Yeckel CW, Volpi E, Wolf SE, Morio B, Chinkes DL, Paddon-Jones D & Wolfe RR (2004). Postexercise protein metabolism in older and younger men following moderate-intensity aerobic exercise. *Am J Physiol Endocrinol Metab* **287**, E513–E522.

Stachenfeld NS, Mack GW, DiPietro L, Morocco TS, Jozsi AC & Nadel ER (1998). Regulation of blood volume during training in post-menopausal women. *Med Sci Sports Exerc* **30**, 92–98.

Takamata A, Ito T, Yaegashi K, Takamiya H, Maegawa Y, Itoh T, Greenleaf JE & Morimoto T (1999). Effect of an exercise-heat acclimation program on body fluid regulatory responses to dehydration in older men. *Am J Physiol Regul Integr Comp Physiol* **277**, R1041–R1050.

Thomas CM, Pierzga JM & Kenney WL (1999). Aerobic training and cutaneous vasodilation in young and older men. *J Appl Physiol* **86**, 1676–1686.

Yang RC, Mack GW, Wolfe RR & Nadel ER (1998). Albumin synthesis after intense intermittent exercise in human subjects. *J Appl Physiol* **84**, 584–592.

Zappe DH, Bell GW, Swartzentruber H, Wideman RF & Kenney WL (1996). Age and regulation of fluid and electrolyte balance during repeated exercise sessions. *Am J Physiol Regul Integr Comp Physiol* **270**, R71–R79.

Acknowledgements

This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, the Ministry of Health, Labour, and Welfare of Japan (Comprehensive Research on Ageing and Health), and from the Japan Society for the Promotion of Science.