Interval walking training and nutritional intake to increase plasma volume in elderly

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ABSTRACT

Purpose: Aerobic training-induced plasma volume (PV) expansion improves thermoregulation, and carbohydrate (CHO) + whey protein supplementation enhanced the effects in older people; however, these were suggested by studies on gym-based cycling training but not on home-based interval walking training (IWT). Moreover, long-term walking training effects on PV remain unknown. **Methods:** Seventeen male and 10 female subjects (~69 yr), having performed IWT for ≥24 months before the study, were used. After pre-intervention measurement (PRE) of PV, plasma albumin content (Alb_{cont}), fasting glucose concentration ($[Glc]_f$), and HbA1c, the subjects were randomly divided into two groups: CHO and Pro-CHO, either consuming CHO (22.5 g) alone or CHO (15 g) + whey protein (10 g), respectively, during additional 5-month IWT from May to November, 2009. After the additional IWT, we measured the same variables again (post-intervention measurement, POST). **Results:** The baseline PV and Alb_{cont} were significantly correlated with the number of IWT days for the 12 months preceding PRE ($r=0.716$, $P<0.001$ and $r=0.671$, $P<0.001$, respectively). In POST, PV and Alb_{cont}, marginally decreased in CHO from the baselines ($P=0.081$ and $P=0.130$, respectively) with increased HbA1c ($P<0.001$) after correction for the baseline $[Glc]_f$ by ANCOVA, but these values remained unchanged in Pro-CHO (both, $P>0.74$), with significant differences in the changes between groups ($P=0.020$, $P=0.041$,and $P=0.018$ respectively). **Conclusion:** PV was proportional to the number of IWT days for 12 months and a CHO + whey protein

supplementation during the 5-month IWT prevented PV reduction for the period of no supplementation, which might be partially linked with blood glucose control mechanisms.

Key words: plasma expansion, interval walking training, carbohydrate and whey protein supplement, long-term training effect, fasting blood glucose, older people.

INTRODUCTION

Plasma volume (PV) expansion through aerobic training has been suggested to improve heat dissipation mechanisms in young people (1-4), but the PV expansion response appear to be attenuated in older subjects (5-7). However, it remains unclear whether the PV response in older people is affected by walking training for several months, longer than that in previous studies (5, 6, 8), due to long-term exercise adaptation mechanisms.

On the other hand, supplementation with a mixture of carbohydrate (CHO) + whey protein during aerobic training at 60-70% of individual peak aerobic capacity ($\dot{V}O_{2peak}$), more than 3 days/week, for several weeks, reportedly enhanced PV expansion with increased albumin content (Alb_{cont}) by causing fluid movement from the interstitial space to the plasma space according to the colloid osmotic pressure gradient to increase PV in older people (5, 6, 8). However, these results were obtained from subjects who performed aerobic training using cycle ergometers in a gym and no studies have been conducted to examine whether the same effects were obtained by home-based walking training which can be performed easily by middle-aged and older people.

One reason that no studies have examined these issues mentioned above is the lack of a tracking system to assess whether exercise intensity, duration, and frequency for a given period reach the level required to increase PV during home-based walking training for several months in older people. In the present study, we used the system that we developed to determine $\dot{V}O_{2peak}$ for walking and to monitor exercise intensity during interval walking training (IWT), repeating a set of fast walking at > 70% $\dot{V}O_{2peak}$ and slow walking at ~40% $\dot{V}O_{2peak}$ for 3 min each, > 5 sets/day, > 4 days/week, and to transfer the data to a server computer through the internet (9, 10). Using this system, we reported that IWT for 5 months increased thigh muscle strength by >10% and $\dot{V}O_{2peak}$ by ~10% (11), with improved symptoms of lifestyle-related diseases (12). However, it is not clear whether PV and Alb_{cont} are increased by IWT and whether the responses are enhanced by a mixture of CHO and whey protein supplementation.

Therefore in Study 1, we examined the hypothesis whether the baseline PV and plasma albumin content (Alb_{cont}) in individuals were associated with the number of IWT days for 12 months preceding the measurement. In Study 2, we examined, in the same subjects as in Study 1, the hypothesis whether a mixture of carbohydrate (CHO) + whey protein supplementation during an additional 5-month IWT would enhance increases in PV and Alb_{cont}. Furthermore, since fasting blood glucose concentration ($[Glc]_f$) in the baseline is known to reflect insulin sensitivity in the peripheral tissue (13) and since improved insulin sensitivity during a long-term exercise adaptation would be involved in PV expansion through improved albumin synthesis in the liver (14, 15), we examined the hypothesis whether there were any association of $[Glc]_f$ with PV and $Alb_{cont,}$ in the baselines for Study 1 and also with changes in PV (ΔPV) and Alb_{cont} (ΔAlb_{cont}) after the additional 5-month IWT for Study 2.

If we obtain results that support the hypotheses, the regimen may be broadly accepted by middle-aged and older people who wish to increase PV to improve heat dissipation mechanisms (5-7) and prevent heat illness, of which incidence is reportedly higher in middle-aged and older people than in young people during midsummer in Japan (16).

METHODS

Subjects and Grouping:

Fig. 1 shows a timeline of the present study. The procedure in this study was approved by the Institutional Review Board for Human Experiments, Shinshu University School of Medicine. The subjects were recruited from those who had performed IWT for more than 24 months in the "Jukunen Taiikudaigaku" project, which is a health promotion program for middle-aged and older people in Matsumoto City. For the recruitment, we displayed a poster in a local community office that the participants visit regularly and distributed leaflets. After the experimental protocol was fully explained, 27 of 30 responders provided written informed consent and enrolled in the study. After the pre-intervention (PRE) measurement to determine the baseline values, we randomly divided the subjects (17 males and 10 females, 57-77 years old) into two groups: CHO (8 males and 5 females) and Pro-CHO (9 males and 5 females), consuming either glucose alone or a mixture of CHO + whey protein during IWT for the next 5 months, respectively. The post-intervention (POST) measurement was performed after completion of the additional 5-month IWT (**Table 1**).

Protocol:

For the PRE measurement, the subjects were invited to a laboratory at 8:00 on the day assigned to individuals from May to June, 2009 after an 11-hr fasting period with free access to water. After conducting interviews on current health status, we measured height, body weight, arterial blood pressure, blood constituents, and PV. On another day within several days after the measurement, the subjects were invited to a gym to measure their peak aerobic capacity ($\dot{V}O_{2peak}$) for walking as described in detail below. The same variables were measured again in the POST measurement within several days after the additional 5-month IWT. In Study 1, we analyzed the variables in the baselines relative to IWT days for 12-month before starting the additional 5-month IWT in individual subjects cross-sectionally. In Study 2, we examined the effects of supplementation during the additional 5-month IWT on the variables longitudinally.

Interval walking training:

Subjects were instructed to repeat more than 5 sets of fast (>70% VO_{2peak}) and slow (~40% $\dot{V}O_{2peak}$) walking for 3 min each, more than 4 days/week. Training intensity was monitored with a tri-axial accelerometer (JD Mate; Kissei Comtec, Matsumoto, Japan) carried on the midclavicular line of the right or left waist. A beeping signal alerted subjects when a change of intensity was scheduled and another melody notified them when their walking intensity had reached the target level every minute. Every 2 weeks, the subjects visited a local community office and the walking record from the tracking devices was transferred to a central server at the administrative center through the internet for automatic analysis and reporting. Trainers used the reports on exercise intensity and other parameters (**Table 2**) to instruct the subjects how best to achieve the target levels. The additional 5-month IWT in Study 2 was performed between May 16th and November 4th, 2009, during which period the daily average atmospheric temperature (T_a) ranged from 3.2 to 27.9°C and the relative humidity (RH) varied between 38% and 91%.

Supplements:

The CHO supplement (90 kcal) was composed of 22.5 g glucose, 0 g protein, 0 g fat, and 43 mg sodium (Weider in jelly multivitamin, Morinaga, Tokyo, Japan). The CHO + whey-protein supplement (100 kcal) was composed of 10 g whey-protein and 15 g CHO mixture of fructose, dextrin, glucose and maltose with 0 g fat and 10 - 30 mg sodium (JogMate, Otsuka, Tokyo). We adopted the supplement since we reported that the supplement intake after daily exercise during a 2-month cycling training increased PV and Alb_{cont} in older people (5, 6).

Dietary intake:

Subjects in both groups were instructed to maintain their dietary habits, except for the supplements, during Study 2. In addition, they were instructed to consume the assigned supplement within 30 min after daily exercise since albumin synthesis in the liver was enhanced during the period (17), but they were not allowed to consume any other foods or fluids except for water more than 60 min before and after exercise each training day. They were also instructed to report foods consumed for 7 consecutive days between May and July 2009 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, 12 subjects in the CHO group and 13 subjects in the Pro-CHO group consumed 1983 ± 145 and 1849 ± 119 kilocalories with diet; 252 ± 10 and 253 ± 15 g carbohydrates, 77 ± 6 and 67 ± 5 g protein, 66 ± 7 and 56 ± 4 g fat, and 4626 ± 520 and 3812 ± 453 mg sodium per day, respectively, with no significant differences between the groups (P

> 0.24), which met the recommended dietary allowances (RDA) for active, older Japanese adults, except for a relatively high sodium intake (18).

Measurements:

$\dot{V}O_{2peak}$ and $\dot{V}O_2$ during IWT measurements with calorimeter:

We determined $\dot{V}O_{2peak}$ by measuring energy expenditure with the accelerometer during graded intensity walking on a flat floor at a slow, moderate, and fast pace for 3 min each, as reported previously (12). This approach was reported to show good agreement with a graded cycling test (19). Also, we determined the oxygen consumption rate ($\dot{V}O_2$) during IWT using the calorimeter. Since the meter is equipped with not only a triaxial-accelerometer but also a barometer, we can estimate energy expenditure precisely even when subjects walk on an incline using a logic that we developed (19).

Arterial blood pressures at rest:

We measured systolic (SBP) and diastolic (DBP) blood pressures of the subjects using the auscultation method 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) after sitting at rest more than 10 min in a room controlled to a T_a of ~28°C and RH of ~50%.

PV and blood constituents:

On the day before the day of PRE and POST measurements, subjects were asked to eat a standardized breakfast and lunch at 07:00 and 12:00, respectively, and to finish the standardized dinner by 21:00. Food was controlled over the course of the day (i.e., standardized breakfast, lunch, and dinner): total calories were ~2100 kcal, total carbohydrates were ~330 g, total protein ~67 g, total fat ~56 g, and sodium ~2.4 g before and after training in the CHO and Pro-CHO groups. The subjects reported to the laboratory at 08:00 normally hydrated but without having eaten any food for at least ~11 h before the measurement. To ensure that they were appropriately 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 a 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 the subjects rested in a sitting position for 30 min, the PV was determined using the Evans blue dye dilution method (7, 20). 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) 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), hemoglobin concentration ([Hb], sodium lauryl sulfate hemoglobin method; Sigma Chemical, St Louis, MO) in triplicate, and hemoglobin A1c (HbA1c, DM-Jack II; Kyowa Medex, Tokyo, Japan). The remaining aliquot of the sample was transferred to a heparin-treated tube and centrifuged at 4°C for 30 min. The separated plasma was used to determine the total plasma protein ([TP]) by refractometry, plasma albumin ([Alb]) concentrations by the bromcresol green method (Wako Chemical, Tokyo), and $[Glc]_f$ (YSI 2300 Stat Plus; Yellow Springs, OH). Alb_{cont} was calculated as a product of PV and [Alb].

Analyses:

Study 1:

We analyzed PV, Alb_{cont}, and $[Glc]_f$ in the PRE measurement relative to the number of IWT days for 12 months, April 2008 - March 2009, using 17 male and 10 female subjects before starting the additional 5-month IWT for Study 2.

Study 2:

We analyzed any significantly different effects of the supplements, CHO or Pro-CHO, during the additional 5-month IWT from May to November, 2009, on the variables by comparing the PRE and POST measurements between the groups using 7 male and 5 female subjects in the CHO group and 9 male and 5 female subjects in the Pro-CHO group, since one male subject was absent from the POST measurement (**Fig. 1**).

Statistics:

A two-way [one-between Supplements (CHO vs. Pro-CHO) and one-within Time (pre vs. post additional 5-month IWT)] ANOVA for repeated measures was used to examine any significant differences in variables (**Table 1**). A one-way ANOVA was used to examine the effects of supplements (CHO vs. Pro-CHO) on the training achievement (**Table 2**). The standard regression analysis was used to analyze the relationships between IWT-days vs. PV and Alb_{cont} (**Fig. 2 A, B**) and between Alb_{cont} vs. PV (**Fig. 2 C**) in Study 1. The analysis was also used to determine the relationships between $[Glc]_f$ vs. PV and Alb_{cont} after pooling the values in the PRE and POST measurements **(Supplemental Fig. 1 A, B**) and also between the baseline $[Glc]_f$ vs. changes in PV (ΔPV), Alb_{cont} (ΔAlb_{cont}), and HbA1c (ΔHbA1c) after the additional 5-month IWT in Study 2 (**Fig. 3 A, B, C).** When we examined the effects of supplements (CHO vs. Pro-CHO) on ΔPV, ΔAlb_{cont}, and ΔHbA1c after the additional 5-month IWT in Study 2, we corrected the changes using an analysis of covariance (ANCOVA) with the baseline $[Glc]_f$ as a covariate **(Fig. 4 A, B, C)** after confirming that the $[Glc]_f$ significantly affected ΔPV, ΔAlb_{cont}, and ΔHbA1c **(Fig. 3 A, B, C).** Similarly, we determined the relationship between ΔAlb_{cont} and ΔPV after the correction (**Fig. 3 D**). The statistical power (1-β) is presented in the text at $\alpha = 0.05$ when the variables were significantly different between the CHO and Pro-CHO groups. All values are expressed as the means ± SE. The null hypothesis was rejected at $P<0.05$.

RESULTS

Table 1 shows the physical characteristics, blood pressures, and plasma constituents of the subjects in Study 1 & 2. The values in Study 1 were from the PRE measurement in all subjects, and the values in Study 2 were presented from the PRE and POST measurements in the CHO and Pro-CHO groups, respectively. We observed a significant increase of HbA1c only in the CHO group (PRE vs. POST, $p = 0.004$). There were no significant differences in other variables – age, height, BMI, $\dot{V}O_{2peak}$, HR_{peak}, SBP, DBP, PV, [TP], [Alb], and $[Glc]_f$ – before and after the additional 5-month IWT in each group or between groups (all, $P > 0.08$), with no interactive effects of [Supplements (CHO vs. Pro-CHO) x Time (PRE vs. POST measurement)] on the variables (all, $P > 0.07$).

As shown in **Fig. 2**, there was a positive correlation between the baseline PV vs. the number of training days for 12 months preceding the PRE measurement (male (M): $r = 0.670$, $P = 0.006$, female (F): $r = 0.808$, $P = 0.005$, Total: $r = 0.716$, $P < 0.001$) [A] and Alb_{cont} (M: $r = 0.509$, $P = 0.053$, F: $r = 0.882$, $P = 0.001$, Total: $r = 0.671$ $P < 0.001$) [B] with a high correlation between PV (y) and Alb_{cont} (x) in all subjects with a regression equation of $y = 21.5x + 2.5$ ($r = 0.930$, $P < 0.001$) [C]. On the other hand, there were no significant correlations between the baseline $[Glc]_f$ or HbA1c vs. the number of training days for 12 months (both, $P > 0.2$).

In addition, the baseline $[Glc]_f$ was negatively correlated with the baseline PV (r = -0.445, P = 0.020) and Alb_{cont} (r = -0.393, P = 0.042) in the PRE measurement. When the values in the PRE and POST measurements were pooled, $[Glc]_f$ was also negatively correlated with PV (r = -0.460, P = 0.001) and Alb_{cont} (r = -0.384, P = 0.004) (**Supplemental Fig. 1 A, B**). [B]. No other variables in **Table 1** were significantly correlated with PV and Alb_{cont} (all, P > 0.1).

Table 2 shows IWT achievements for 12 months preceding the PRE measurements in Study 1 and the achievements of the CHO and Pro-CHO groups in Study 2. As shown in the table, the number of IWT days per week in both groups for Study 2 was significantly higher than those in Study 1 (both, P < 0.01), but there were no significant differences in these variables between the groups (all, P > 0.443).

As in **Fig. 3,** the baseline $[Glc]_f$ in the PRE measurement was significantly correlated with ΔPV [A], ΔAlb_{cont} [B], and ΔHbA1c [C] after the additional 5-month IWT in Study 2. Therefore, we determined the relationship between ΔPV and ΔAlb_{cont} after correcting for the $[Glc]_f$ by ANCOVA. We found that ΔPV (y) was highly correlated with ΔAlb_{cont} (x) with a regression equation of $y = 22.5\,x - 0.1$ (r = -0.960, P < 0.001) [D].

As in **Fig. 4 A, B,** we found that PV and Alb_{cont} corrected for the $[Glc]_f$ by ANCOVA marginally decreased from the baselines in the CHO group (P = 0.081 and P = 0.130, respectively), but they remained unchanged in the Pro-CHO group, with significant differences in the changes between groups (P = 0.020, 1-β = 0.621 and P = 0.041, 1-β = 0.512, respectively).

In addition, we found that ΔHbA1c after correcting for the $[Glc]_f$ by ANCOVA increased in the CHO group ($P < 0.001$) and remained unchanged in the Pro-CHO group with a significant difference between groups ($P = 0.018$, $1-\beta = 0.687$).

DISCUSSION

In the present study, we found that the baseline PV and Alb_{cont} were proportional to the number of IWT days for 12 months preceding the PRE measurement, and they were negatively correlated with the baseline $[Glc]_f$. In addition, we found that CHO + whey-protein supplementation during the additional 5-month IWT prevented the marginal reductions in PV and Alb_{cont} and the increase in HbA1c in the CNT group for Study 2. These changes were significantly different between groups after correction for the baseline $[Glc]_f$ by ANCOVA.

Subjects:

The BMI, height, and $\dot{V}O_{2peak}$ values reported in this study (**Tables 1**) were similar to those previously reported in age-matched Japanese populations (12, 21, 22), but $\dot{V}O_{2peak}$ was slightly higher in this study population than those populations, probably because our subjects had performed IWT for more than 24 months before participating in the present study. Therefore, the characteristics of the subjects in this study generally reflected those of this age group of the Japanese population.

PV expansion vs IWT achievements for 12 months:

In contrast to the results in the previous studies (5-7) reporting that aerobic training using the cycle ergometer for 8 and 18 weeks caused no PV expansion in older subjects, we found in the present study that the baseline PV and Alb_{cont} were proportional to the number of IWT days for 12 months preceding the PRE measurement (**Fig. 2 A, B**) with a high correlation between PV and Alb_{cont} (**Fig. 2 C**). In addition, we found that $[Glc]_f$ was inversely correlated with PV and Alb_{cont} in the PRE measurement, and moreover, this was confirmed when the values in the PRE and POST measurements were pooled (**Supplemental Fig. 1 A, B**). These results suggest that IWT for several months, longer than the several weeks employed in previous studies (5, 6), increases PV and that glucose metabolism is involved in this response.

The detailed association of PV and Alb_{cont} with $[Glc]_f$ in the PRE measurement remained unknown; however, it has been suggested that the higher $[Glc]_f$ is suggestive of the lower insulin sensitivity in the peripheral tissues (13). Since insulin is known to stimulate the albumin synthesis rate in the liver (14, 15), the lower sensitivity in subjects with higher $[Glc]_f$ might attenuate PV expansion by aerobic training. As $[Glc]_f$ likely decreases with an increased number of IWT days – although we found no direct and significant correlations between them most likely because of the high inter-individual variation in $[Glc]_f$ – the significant correlations between the number of IWT days vs. PV and Alb_{cont} in **Fig. 2** might be associated with improved glucose metabolism attained by IWT for several months. This idea might be supported by the results in Study 2.

PV expansion by 5-month IWT with supplementation:

We conducted Study 2 to examine the effects of CHO + whey protein supplementation immediately after daily IWT for the additional 5 months. Five months was chosen for IWT because the improvements in physical fitness and in the symptoms of lifestyle-related diseases, including $[Glc]_f$ and HbA1c, appeared after this period (11, 12). As a result, there were no significant differences in ΔPV, ΔAlb_{cont}, except for the increase in HbA1c on the CHO group (**Table 1**); however, when corrected for the baseline $[Glc]_f$ by ANCOVA after confirming that the $[Glc]_f$ was significantly correlated with the changes **(Fig. 3**), we found that PV and Alb_{cont} marginally decreased with an increase in HbA1c in the CHO group whereas they remained unchanged in the Pro-CHO group. We also found that ΔPV was highly correlated with ΔAlb_{cont} with a similar regression coefficient to that in **Fig. 2 C** (**Fig. 3 D**). Significant differences in ΔPV, ΔAlb_{cont}, and ΔHbA1c were evident between the two groups **(Fig. 4**), while we failed to detect any significant difference in $\Delta[Glc]_f$, probably due to high inter-individual variation. These results suggest a close association between PV expansion and glucose metabolism through increased Alb_{cont} in older subjects.

As for the possible association between PV and glucose metabolism, Masuki et al. (23)

recently examined the effects of milk product supplementation during IWT for 5 months in older women who had performed IWT for more than 6 months before participating in the study, almost equivalent to the Pro-CHO group in the present study, and suggested that activity (methylation) of pro-inflammatory cytokine genes such as *NFKB1* and *NFKB2* in leukocyte were suppressed (enhanced) by the supplementation. Since chronic inflammation with aging has been suggested to be one of the major causes of lifestyle-related diseases including diabetes mellitus (24), no reduction in PV and Alb_{cont} in the Pro-CHO group in **Fig. 4** might be at least in part caused by the suppression of chronic inflammatory responses to enhance insulin sensitivity in the liver.

The detailed mechanisms for the marginal decreases in PV and Al_{bcont} and the significant increase in HbA1c after the additional 5-month IWT in Study 2 for the CHO group (**Fig. 4**) remain unknown; however, seasonal variation of PV; decreasing in winter and increasing in summer (25), and seasonal variation of $[Glc]_f$; increasing in winter and decreasing in summer (26) have been suggested. On the other hand, Nakano et al. (27) recently suggested seasonal variation of pro-inflammatory gene (*NFKB2*) activity; most inactivated (most methylated) in April - June and most activated (most demethylated) in October - November. In addition, Masuki et al. (23) suggested in the above study that *NFKB1* and *NFKB2* genes and other related inflammatory genes in the group with no milk product supplementation, equivalent to the CHO group in the present study, were marginally activated (demethylated) after the 5-month IWT from April to September, similar to the period in the present study. These results suggest that the marginal reductions in PV and Alb_{cont} for the CHO group **(Fig. 4 A**, **B**) might link with the seasonal variation of $[Glc]_f$ which can be partially explained by seasonal fluctuation of chronic inflammation levels in the body.

As shown in **Fig. 2**, PV and Alb_{cont} were proportional to the IWT days for 12 months in Study 1 but both marginally decreased after 5 months of IWT in the CHO group for Study 2 as shown in **Fig. 4**. The reasons for the discrepancy at a glance might be that the increases in PV and Alb_{cont} attained by IWT alone had likely reached plateau levels by more than 24-month training, but the levels might be influenced by seasonal fluctuation of glucose metabolism with a chronic inflammation state in the body for some reason. In the present study, we found that a mixture of CHO and whey protein supplementation during IWT prevented these responses.

Limitations:

In the present study, we did not measure thermoregulatory responses. However, Okazaki et al. (5) examined the effects of a mixture of CHO and whey protein intake on PV and thermoregulatory responses during exercise in a hot environment in older men and suggested that the supplement increased PV by ~6% to improve the sweat rate response by 18% and the cutaneous vasodilator response by 89% at a given increase in esophageal temperature. Recently, Kataoka et al. (6) examined the effects of the supplement in hypertensive older men and reported similar results with reduced arterial blood pressure. In the present study, because the difference in the change of PV after the additional 6-month IWT between the CHO and Pro-CHO groups was similar as in previous studies (5, 6), the thermoregulatory responses would be improved in the Pro-CHO group compared with the CHO group as in the previous studies (5, 6).

Conclusions:

PV and Alb_{cont} were proportional to the number of IWT days for 12 months, and a mixture of CHO + whey protein supplementation during IWT for the additional 5 months prevented the marginal reduction in PV and Alb_{cont} in the supplementation of CHO alone, which might be partially linked with blood glucose control mechanisms.

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CONFLICT OF INTEREST

No conflicts of interest, financial or otherwise, are declared by the authors. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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FIGURE LEGENDS:

Fig. 1:

A timeline of the experiment. CHO, the carbohydrate group; Pro-CHO, the carbohydrate + whey protein group. IWT, interval walking training; Study 1, a retrospective study on the relationship between plasma volume (PV) and training days from April 2008 to March 2009;

Study 2, a prospective study on the effects of a mixture of CHO and whey protein intake during IWT from May to November, 2009 on PV.

Fig. 2:

Relationships between training days vs. plasma volume (PV) [A] and plasma albumin content (Alb_{cont}) [B], and also between Alb_{cont} and PV [C] in 17 male (M, squares) and 10 female (F, circles) subjects for 12 months from April, 2008 to March, 2009 in Study 1 before starting Study 2.

Fig. 3:

Relationships between fasting plasma glucose in the pre-intervention measurement ($[Glc]_{f,\ pre}$) vs changes in PV (ΔPV) [A], Alb_{cont} (ΔAlb_{cont}) [B], and HbA1c (ΔHbA1c) [C] after additional 5-day IWT in Study 2 with a relationship between ΔAlb_{cont}' and ΔPV', which were corrected by ANCOVA with the baseline $[Glc]_{f,\ pre}$ as a covariate [D]. The open symbols indicate the subjects in the CHO group, and the closed symbols indicate those in the Pro-CHO group.

Fig. 4:

Changes in plasma volume (ΔPV'), plasma albumin content (ΔAlb_{cont}'), and HbA1c (ΔHbA1c') from the baselines before starting additional 5 day-IWT in Study 2 in May, 2009.

These values are corrected by ANCOVA with the baseline plasma glucose concentration $[Glc]_f$ as a covariate. The means and SE bars are presented for 12 and 14 subjects in the carbohydrate (CHO) and the carbohydrate + whey protein (Pro-CHO) groups, respectively. ##, vs the baseline at $P<0.01$. *, vs. the CHO group at $P < 0.05$.

Supplemental Fig. 1

Relationships between fasting plasma glucose concentration ($[Glc]_f$) vs. plasma volume (PV) [A] and plasma albumin content (Ab_{cont}) [B] in 16 male and 10 female subjects before and after additional 5-month IWT for Study 2. The open symbols indicate the subjects in the CHO group and the closed symbols indicate those in the Pro-CHO group. The squares indicate male subjects and the circles indicate female subjects.

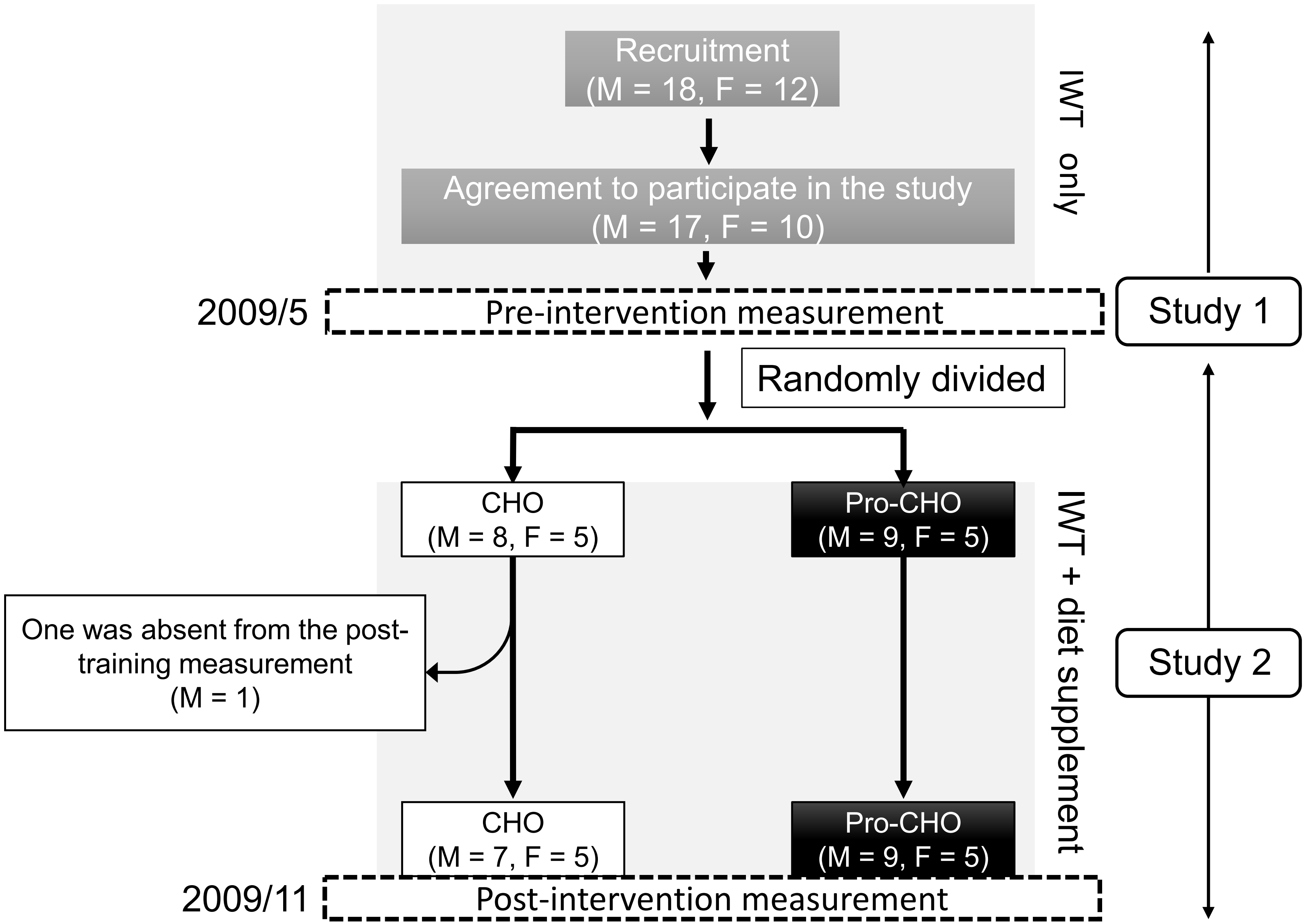


Fig. 1 Uchida et al.

Table 1: Physical characteristics, blood pressures, and plasma constituents of the subjects.

	Study 1	Study 2			
	Total (M = 17, F = 10)	CHO (M = 7, F = 5)		Pro-CHO (M = 9, F = 5)	
		PRE	POST	PRE	POST
Age, yrs	68.2 ± 1.0	69.0 ± 1.6		67.1 ± 1.3	
Height, cm	161 ± 1	160 ± 2		162 ± 2	
Body mass, kg	61.0 ± 1.6	60.2 ± 2.1	60.3 ± 2.1	62.7 ± 2.4	62.1 ± 2.4
BMI, kg/m^2	23.6 ± 0.5	23.5 ± 0.8	23.5 ± 0.8	23.8 ± 0.8	23.6 ± 0.8
$\dot{V}O_{2peak}$, ml/kg/min	25.4 ± 1.1	25.6 ± 2.2	25.2 ± 2.3	25.3 ± 1.1	25.8 ± 1.1
HR_{peak}, beats/min	137 ± 5	134 ± 7	138 ± 6	139 ± 7	136 ± 5
SBP, mmHg	138 ± 3	139 ± 6	140 ± 5	137 ± 4	134 ± 4
DBP, mmHg	79 ± 2	76 ± 3	76 ± 2	83 ± 3	80 ± 2
PV, ml/kg	40.3 ± 1.0	40.2 ± 1.3	38.2 ± 1.4	41.8 ± 1.4	42.1 ± 1.4
[TP], g/dl	6.96 ± 0.09	6.85 ± 0.12	6.97 ± 0.10	7.09 ± 0.13	7.01 ± 0.10
[Alb], g/dl	4.45 ± 0.03	4.44 ± 0.06	4.47 ± 0.06	4.49 ± 0.04	4.47 ± 0.05
$[Glc]_f$, mg/dl	109 ± 3	114 ± 5	111 ± 5	106 ± 4	103 ± 3
HbA1c, %	5.39 ± 0.14	5.60 ± 0.23	5.75 ± 0.22**	5.23 ± 0.18	5.26 ± 0.13

BMI, body mass index; $\dot{V}O_{2peak}$, peak aerobic capacity for walking; HR_{peak}, peak heart rate for walking; SBP and DBP, systolic and diastolic blood pressures, respectively; PV, plasma volume; [TP], total protein concentration in plasma; [Alb], plasma albumin concentration; $[Glc]_f$, fasting plasma glucose concentration in Study 1 and Study 2. The values in Study 1 and pre-intervention (PRE) values in Study 2 are in May to June, 2009 before starting Study 2. The post-intervention (POST) values are in October to November, 2009 after finishing IWT in Study 2. The values are the means ± S.E. **, vs. pre at $P < 0.01$.

Table 2: Training achievement

	Study 1			Study 2					
	Total (M = 17, F = 10)			CHO (M = 7, F = 5)			Pro-CHO (M = 9, F = 5)		
Training days, days/week	3.55	±	0.28	4.17	±	0.47*	4.37	±	0.29*
Fast walking									
Time, min/walking day	25.1	±	1.9	26.0	±	2.7	26.5	±	2.7
Intensity, ml/kg/min	17.8	±	0.8	19.6	±	1.4	18.7	±	0.8
Slow walking									
Time, min/walking day	25.2	±	2.0	28.1	±	5.5	23.6	±	1.6
Intensity, ml/kg/min	11.0	±	0.5	10.5	±	0.9	11.3	±	0.7

The training achievements in Study 1 are from April, 2008 to March 2009 and those in Study 2 are from May to October, 2009. The values are the means ± S.E. *, vs. Study 1 at $P < 0.05$.

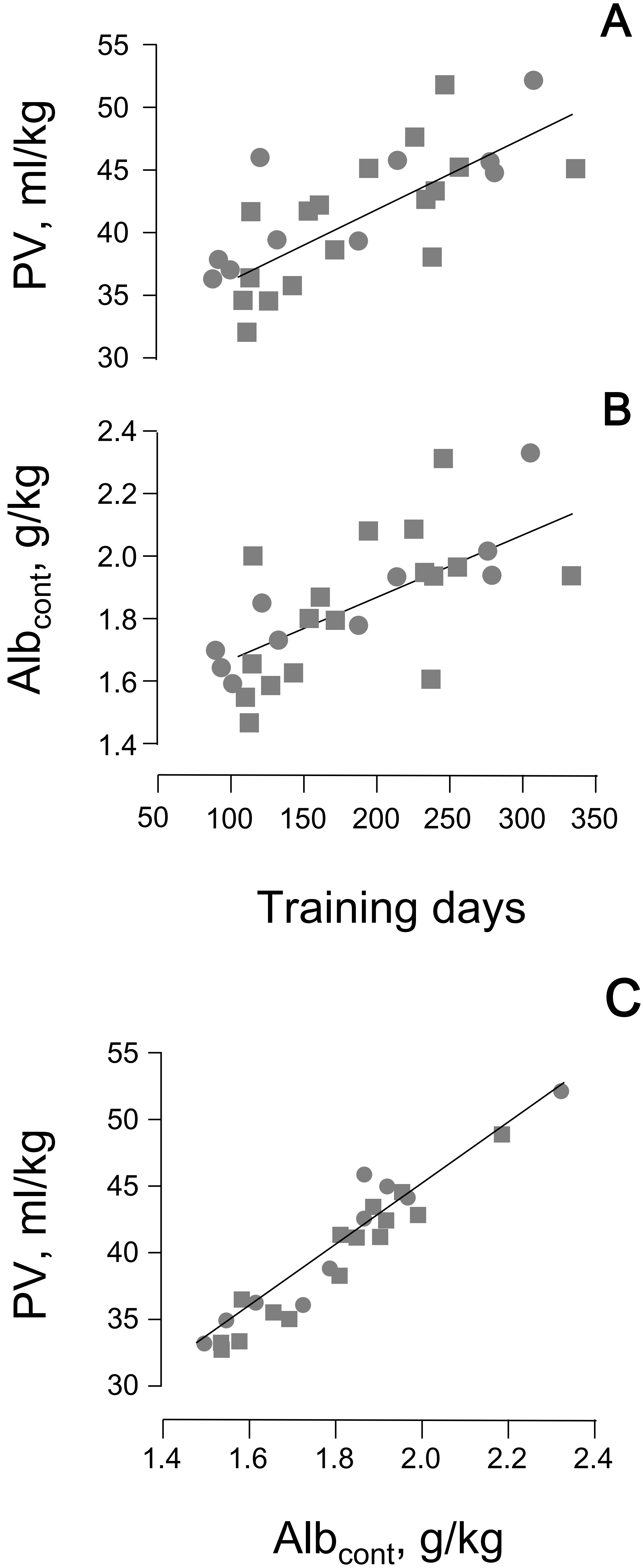


Fig. 2. Uchida et al.

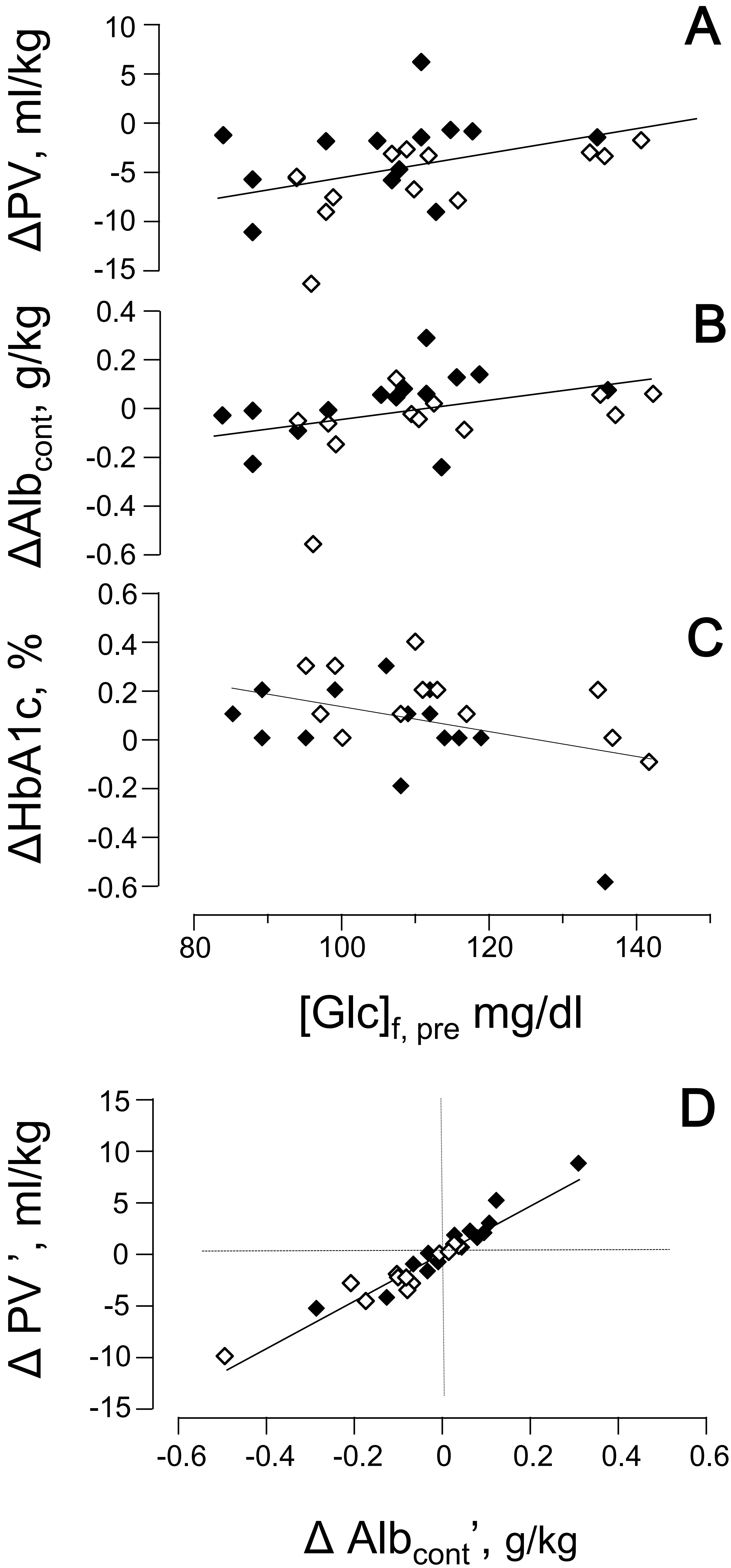


Fig. 3. Uchida et al.

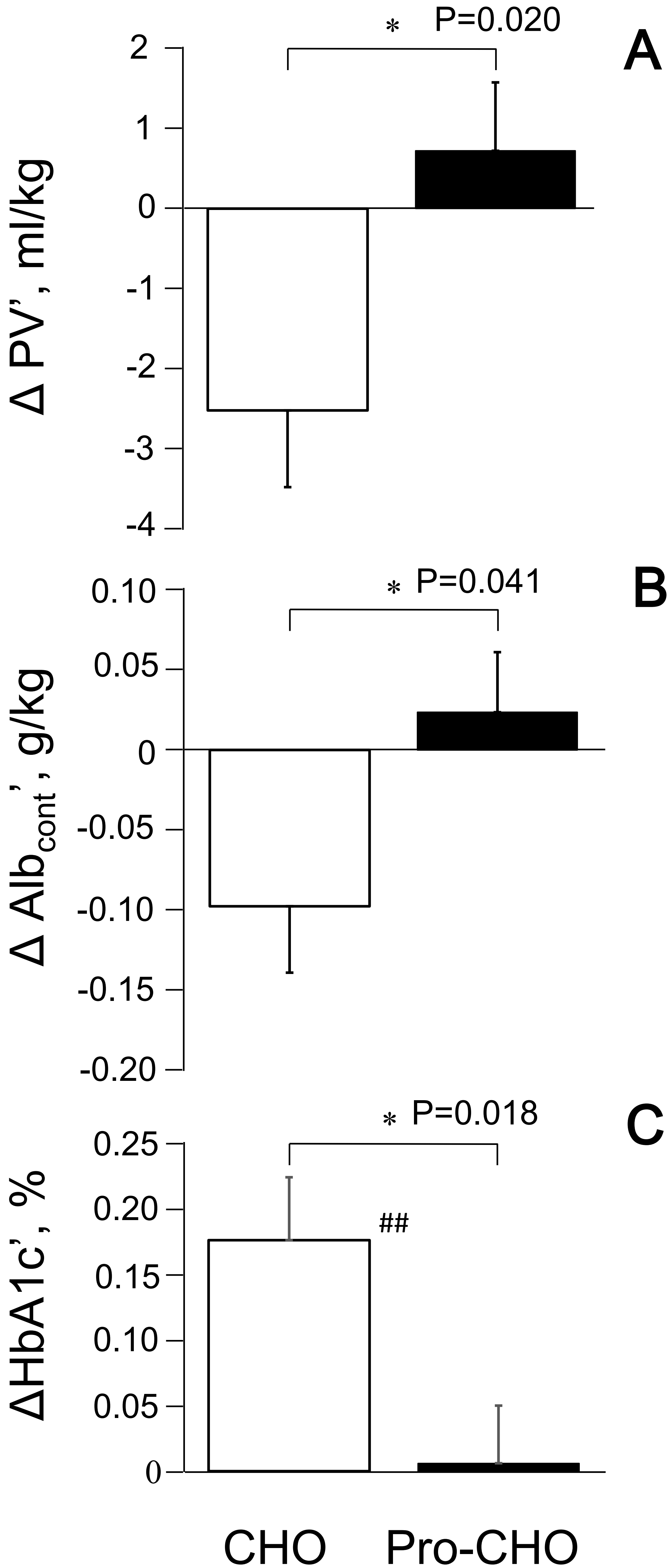
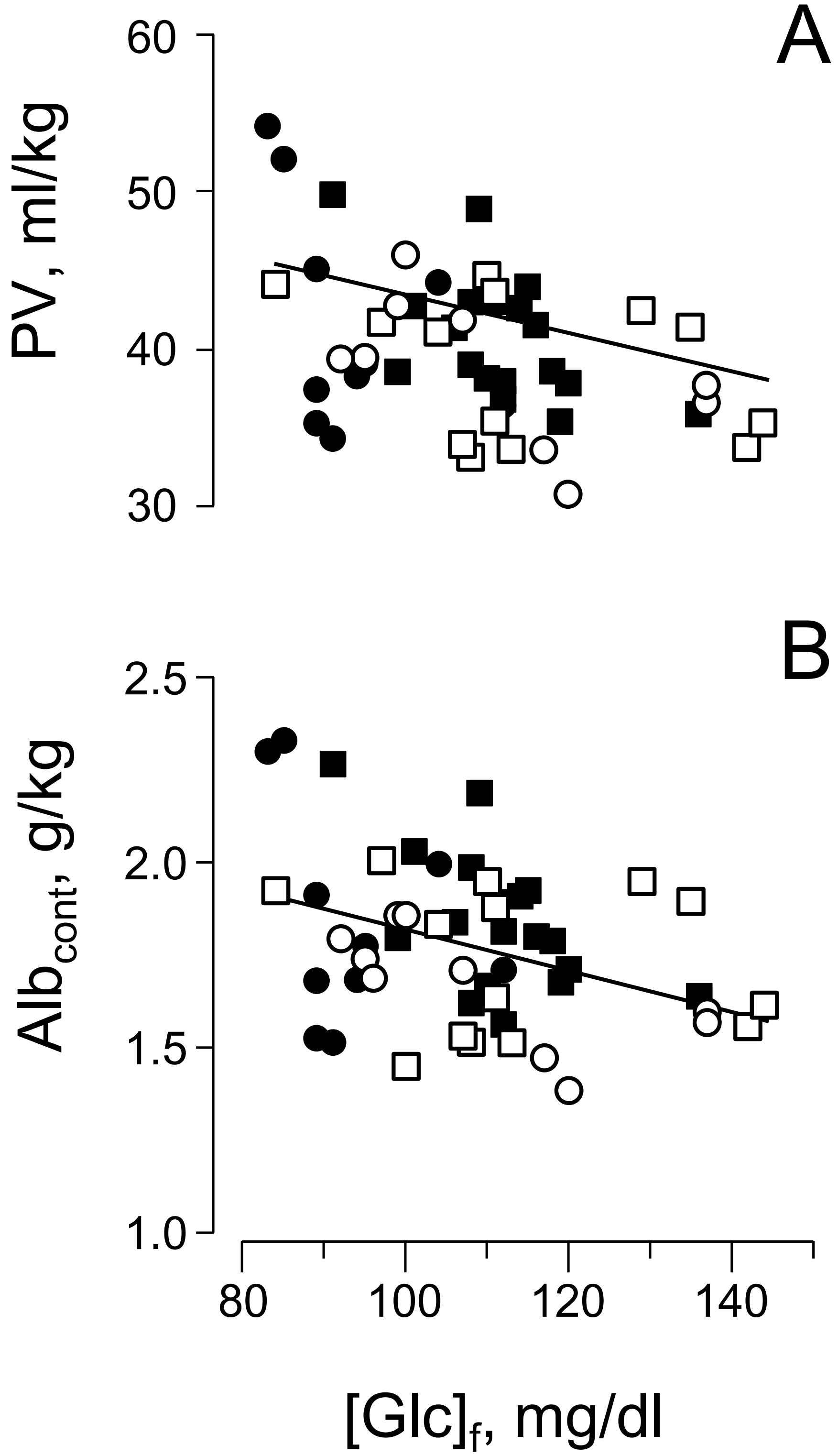


Fig. 4 Uchida et al.



Supplemented Fig.   Uchida et al.