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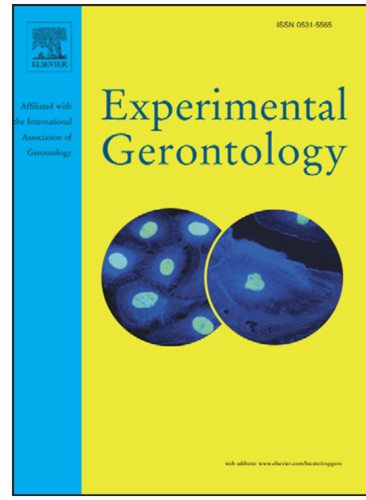

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Effects of mild calorie restriction and high-intensity interval walking in middle-aged and older overweight Japanese

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Running Title: Mild diet and exercise for overweight

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Abbreviations: CR, calorie restriction; fGlc, fasting plasma glucose; HDL-C, HDL-cholesterol; HIW, high-intensity interval walking; HR_{max}, maximal heart rate; MCR, mild calorie restriction; LDL-C, LDL-cholesterol; TC, total cholesterol; TG, triglycerides; VO_{2peak}, peak aerobic capacity for walking.

We investigated whether a combination of mild calorie restriction (MCR) and high-intensity interval walking (HIW) improved physical fitness more than HIW alone in middle-aged and older overweight Japanese (40-69 years old, BMI ≥ 23.6 kg/m^2). Forty-seven women and 16 men were divided into MCR+HIW and HIW groups. All subjects performed HIW: ≥ 5 sets of 3-min low-intensity walking (40% peak aerobic capacity for walking, VO_{2peak}) and 3-min high-intensity walking (≥ 70% VO_{2peak}) per day, ≥ 4 days per week, for 16 weeks while energy expenditure was monitored with a tri-axial accelerometer. The MCR+HIW group consumed meal replacement formula (240 kcal): a mixture of low-carbohydrates and -fat and high-protein, for either lunch or dinner everyday and therefore had ~87% of the energy intake of the HIW group during the intervention period. Although the HIW group showed improvements in BMI, blood pressure, and several blood chemicals, the MCR+HIW group had greater improvement. Moreover, the medical expenditure for the 6 months including the intervention period was 59% lower in the MCR+HIW group than in the HIW group. Our strategy of a short-term combination of MCR and HIW may thus prevent lifestyle-associated diseases and improve health in middle-aged and older overweight Japanese.

1. Introduction

In Japan, the number of overweight and obese middle-aged and older people has been increasing yearly (Kubo et al., 2003, Kuriyama et al., 2004), accompanied by an increased incidence of metabolic syndromes such as hypertension, type II diabetes, hyperlipemia, and atherosclerosis (Ishikawa-Takata et al., 2002; Hasegawa et al., 2005; Zhang et al., 2005; Shiraishi et al., 2006; Arai et al., 2006). Indeed, it has been suggested that the risk for incidence of metabolic syndrome is increased in Japanese people with a BMI above 25.0 kg/m^2 (Matsuzawa et al., 2000; Japan Society For The Study Of Obesity (JASSO), 2002), and, in fact, the annual medical expenditure among Japanese with BMI $\geq$ 25.0 kg/m^2 was reported to be higher than that of Japanese with BMI < 24.9 kg/m^2 (Kuriyama et al., 2002). This may be a result of recent increased in westernized dietary habits and a sedentary lifestyle (Hawks et al., 2003; Watanabe et al., 2004; Arai et al., 2006; Maskarinec et al., 2006; Otsuka et al., 2006).

Calorie restriction (CR) and exercise training have been recommended as countermeasures against the development of overweight and obesity. CR has been suggested to have multiple beneficial effects, including increased physical fitness, vitality, and resistance to oxidative stress (Bluher et al., 2003; Cohen et al., 2004; Cox et al., 2004; Rodgers et al., 2005). Exercise has been suggested to significantly contribute to body weight control (Schoeller, 1998; Bhattacharya et al., 2005; Kretschmer et al., 2005; Kondo et al., 2006; Redman et al., 2007) as well as to increase physical fitness in obese animals and humans (Novelli et al., 2004; Rector et al., 2004; Albeck et al., 2006; Cuntinho et al., 2006; Huang et al., 2006), and moreover, combined treatment with CR and exercise has been suggested to be more effective in maintaining a low body weight with low fat deposition than CR alone (Couturier et al., 2004; Kretschmer et al., 2005).

However, several studies have not provided support for the merits of combined treatment, suggesting that the effects of combined exercise training and CR are not greater than the effects of either alone (Byner et al., 1999; Cox et al., 2003; Colak and Ozcelik, 2004; Hioki et al., 2004; Nicklas et al., 2004; Giannopoulou et al., 2005). However, in nearly all of these studies were conduced using severely obese people with BMI ≥ 30 kg/m^2 who underwent high intensity exercise training involving weight training, cycling, and running and severe CR with energy intake ≤ 75% of a standard diet (Colak and Ozcelik, 2004; Hioki et al., 2004; Nicklas et al., 2004; Giannopoulou et al., 2005), and lasting more than 6 months in western countries.

In Japan, it may be difficult for middle-aged or older overweight people to perform such high-intensity exercise combined with severe CR in their daily activity since this population requires medical supervision to avoid injuries during exercise and nutritional disorders during CR. Moreover, the population of highly obese people is only 2.0-3.0% of the total population in Japan (Yoshiike et al., 1998; Anuurad et al., 2003). Thus, it remains unknown whether mild exercise training combined with mild CR (MCR) is more effective in the prevention of lifestyle-associated diseases and deterioration of physical fitness than mild exercise training alone in middle-aged and older overweight people.

To examine this, we subjected subjects to MCR by replacing either regular lunch or dinner with a meal replacement formula with very low carbohydrate and fat content, and high protein, and sufficient minerals and vitamins (Stordy, 1989; Bryner et al., 1999; Raghuwanshi et al., 2001; Miyashita et al., 2004), everyday, which has been suggested to improve physical fitness in middle-aged obese women (Okura et al, 2007). Also, we used high-intensity interval walking (HIW) as mild exercise training, which has been suggested to increase thigh muscle strength and peak aerobic capacity (VO_{2peak}) by

10-20% with ~10 mmHg and 5 mmHg reductions in systolic and diastolic blood pressure, respectively, after 5-months of training whereas these changes were minimal after moderate-intensity continuous walking exercise training (Nemoto et al., 2007). Therefore, in the present study, we hypothesized that a combination of MCR and HIW training might be effective for improving physical fitness in overweight middle-aged and older people.

2. Subjects and Methods

2.1. *Subjects*

We recruited middle-aged and older (40-69 years old), overweight and obese (BMI ≥ 23.6 kg/m^2), female and male Japanese volunteers from the community based health promotion program Jukunen Taiiku Daigaku Program in Matsumoto. This program was organized to promote health and wellness for middle-aged and older people through HIW training since 2003. Although overweight for Japanese is categorized as 23.0 ≤ BMI < 25.0 (kg/m^2) (JASSO, 2002; Anuurad et al., 2003), we excluded subjects with BMI 23.0–23.5 kg/m^2 because we were concerned that we would have to stop CR in some MCR+HIW subjects with BMI 23.0–23.5 kg/m^2 when their BMIs decreased to the normal range of 18.5-22.9 kg/m^2 (JASSO, 2002; Anuurad et al., 2003) before the end point of the intervention.

2.2. *Design*

As shown in **Fig. 1**, to recruit subjects for the present study, we verbally explained to participants in the Jukunen Taiiku Daigaku Program the protocol and significance of this study using an explanatory leaflet while hearing their medical history. The exclusion criteria were 1) presence of osteoarthritis, knee pain, or pregnancy; 2) current treatment with a low calorie diet; or 3) contraindications for an exercise program (stroke, serious hypertension, serious infectious disease, serious inflammation, type I diabetes, renal disease, or liver disease). After 2 more weeks, we obtained informed consent from the 80 subjects (60 females and 20 males) in the study in compliance with the Ethical Committee's Review Board on Human Experiments, Shinshu University School of Medicine.

Then, we measured height, body weight, blood pressure, pre-training VO_{2peak}, maximum heart rate (HR_{max}), and isometric knee extension and flexion force, and took blood samples after an overnight fast with free access to water in all subjects within a few weeks after April 1, 2005. For blood biochemical parameters at baseline, we measured serum LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), total cholesterol (TC), triglycerides (TG), and plasma glucose (fGlc) concentration.

After confirming that subjects were accustomed to HIW and had mastered the use of a tri-axial accelerometer (Jukudai Mate, Kissei Comtec, Matsumoto, Japan) for 2 weeks as described below, the 80 subjects were divided into smaller groups of 33 (24 females and 9 males) for the HIW group and 47 (36 females and 11 males) for the MCR+HIW group so as not to have any significant differences in the baseline between the groups.

After learning how to perform HIW and also how to record their daily diet and beverage intake in a diary provided by dieticians, all subjects started HIW training shortly after May 9 and continued for the subsequent 16 weeks. Subjects in the MCR+HIW group replaced either their lunch or dinner with a meal replacement formula every day. We received daily records of HIW training and food intake as recorded in the diaries from all subjects every 2 weeks when they visited a local community office to have their body weight and blood pressure measured as detailed below. Subjects were instructed to continue to take all the medications prescribed by their family doctors during the intervention.

Within 2 weeks after the end of the 16-week intervention in September, we measured isometric knee flexion and extension force and took blood samples again in all subjects. We also investigated total medical expenditure for the 6 months from April 1 to September 30, 2005 in 37 subjects (HIW, 17; MCR+HIW, 20) who were enrolled in the national health insurance system.

2.3. Measurements

Blood pressure:

Blood pressure was measured by nurses using the auscultation method after a 10-min rest in a sitting position at ~25 ºC room temperature.

Knee force:

Isometric knee extension and flexion force was measured on the dominant leg with an isometric force meter (Isoforce GT330, OG Giken, Okayama).

VO_{2peak}:

Before HIW training, we determined pre-training VO_{2peak} with a graded walking exercise (Nemoto et al., 2007). Subjects were equipped with the accelerometer on the midclavicular line of their left- or right-side waist. After 3 min of baseline measurements were made at rest, subjects walked for 3 min on a flat floor at 3 graded subjective velocities (slow, moderate, and fast) while 3-dimensional acceleration and heart rate were recorded. VO_{2peak} and HR_{max} were adopted as the averages for the last 30 seconds of 3-min maximal walking velocity. The square root of summed 3 dimensional acceleration from the accelerometer was transferred to a computer and converted to VO_{2peak}. Seventy % of VO_{2peak} was programmed in an accelerometer as the target level for HIW for individuals.

2.4. HIW training

Subjects were told to repeat the following regimen 4 or more times per week: 5 or more sets of 2- to 3-min low-intensity walking intervals (at ~40% of the pre-training VO_{2peak}), followed by 3 min of high-intensity walking (≥70% but <85% VO_{2peak}). The intensity and numbers of steps were monitored with the accelerometer carried on the midclavicular line of subjects' left- or right-side waist. During training, a beeping signal alerted subjects when a change of intensity was scheduled and another beeping signal let them know every minute whether they had accomplished high-intensity walking for the preceding 1 min. They performed this program at a self-chosen time each day. Every 2 weeks, subjects visited a local community office, and the tracking devices' data were transferred to a central server at an administrative center through the internet for automatic analysis and reporting. Trainers used these reports of tracked daily exercise intensity and the other parameters in **Table 1** to instruct subjects on how best to achieve the target levels. If targets were not met, the trainers encouraged them to increase their efforts to achieve them. Atmospheric temperature was 15.3-24.5˚C and relative humidity was 56-72% during intervention.

2.5. MCR

We used a meal replacement formula (MicroDiet-S, Sunny Health Co., Ltd., Tokyo) following the very low calorie diet theory (Stordy, 1989). The meal replacement formula was a low calorie food (240 kcal/meal), which had very low carbohydrate and fat content, was high in proteins, and which provided sufficient minerals and vitamins. The MCR+HIW group consumed one drink daily with a choice of four flavors instead of lunch or dinner and also consumed one pack of cookies as a daytime snack for 16 weeks. Subjects recorded all types and volumes of foods, drinks, fruits, snack foods, and sweets consumed for 3 consecutive days every 2 weeks in their diaries. They were given instruction by dietitians on how to achieve a well-balanced diet based on their food records at the same time as HIW instruction. The subjects in the HIW group also had their food records reviewed by dietitians but were not instructed about a well-balanced diet. We calculated the carbohydrate, protein, and lipid content consumed by subjects everyday with total caloric intake based on the records.

202 *2.6. Grouping of subjects for analyses:*

203 Twenty-three subjects (17 females and 6 males) in the HIW group and 40 subjects (30
204 females and 10 males) in the MCR+HIW group completed the protocol. Fifteen subjects
205 were excluded because they did not visit an administrative center on the 8^{th} or 16^{th} week of
206 training so that we could not assess their compliance to HIW and CR for the preceding 6
207 weeks, and also could not measure their physical fitness and blood pressure at that time.
208 Also, 2 more subjects were excluded because we could not measure their isometric knee
209 force and/or take their blood samples within 2 weeks of the end of intervention. As a
210 result, the numbers of subjects for analysis was 23 (17 females and 6 males) in the HIW
211 group and 40 (30 females and 10 males) in the MCR+HIW group.

212 Furthermore, to investigate the combined effects of MCR and HIW on hypertensive
213 subjects with systolic pressure ≥ 140 mmHg or diastolic pressure ≥ 90 mmHg, subjects
214 were divided into four subgroups. There were 5 (5 female and 0 male) subjects in the
215 hypertensive HIW group, 15 (10 female and 5 male) subjects in the hypertensive
216 MCR+HIW group, 13 (9 female and 4 male) subjects in the normotensive HIW group, and
217 18 (15 females and 3 males) subjects in the normotensive MCR+HIW group, according to
218 the values at baseline.

219 *2.7. Statistical analyses*

220 Before starting this study, we examined any significant differences in age, height,
221 body weight, BMI, blood pressure, isometric knee force, VO_{2peak}, HR_{max}, and blood
222 biochemical parameters between the HIW and MCR+HIW groups with Student's *t* test.
223 Using this test, we also examined any significant differences in average daily energy
224 intake or expenditure, including intake of carbohydrates, proteins, and lipids, HIW
225 exercise time, and total energy expenditure as calculated with the accelerometer.

226 Two-way ANOVA for repeated measures was used to test for any significant
227 differences in BMI, blood pressure, isometric knee force, VO_{2peak}, HR_{max}, or blood
228 chemicals. Subsequent *post hoc* tests to determine any significant differences in various
229 pair wise comparisons were performed using Fisher's protected least significant difference
230 test. Simple linear regression analysis was used to test any significant correlations
231 between changes in BMI and total energy intake or energy consumption by HIW training.
232 All statistical calculations were performed with a statistical software package
233 (STATVIEW J-5.0; SAS Institute Japan Ltd, Tokyo, Japan). Values are presented as
234 means ± SEM, and a P value < 0.05 was regarded as significant.
235 *2.8. Ethics*
236 We certify that the Ethical Committee's Review Board on Human Experiments of
237 Shinshu University School of Medicine approved the ethical use of human volunteers and
238 the protocol in this study.
239

3. Results

3.1. Baseline measurements

As shown in **Table 1**, there were no significant differences in age, height, body weight, and BMI between the HIW and MCR+ HIW groups in either gender before starting HIW training. Subjects' BMI was categorized as mild obesity at 25 ≤ BMI < 30 kg/m^2 according to the criteria for overweight and obesity in Japanese (Matsuzawa et al., 2000; JASSO, 2002) while systolic and diastolic blood pressures in both groups were both below the criteria for hypertension; <140mmHg and <90 mmHg, respectively (Japan Society of Hypertension, 2004).

As shown in **Table 2**, there were no significant differences in blood biochemical parameters between groups at baseline. LDL-C, TC, and TG in both groups were below the criteria for hyperlipidemia < 1.40 g/L for LDL-C, < 2.20 g /L for TC, and < 1.50 g/L for TG (Japan Atherosclerosis Society, 2002). The fGlc concentration in both groups was below the criterion for hyperglycemia, < 1.10 g/L (Alberti and Zimmer, 1998; Matsuzawa, 2005).

As shown in **Table 3**, there were no significant differences in isometric knee forces, HR_{max}, or VO_{2peak} at baseline between groups in either gender.

3.2. Training progress

As shown in **Table 4**, total walking time and total energy expenditure for HIW training was not significantly different between groups. However, time of high-intensity walking per day and total walking time during the intervention were significantly shorter in the MCR+HIW group than in the HIW group.

3.3. Diet components

As shown in **Table 5**, total energy intake in the MCR+HIW group was ~13% less than in the HIW group for both genders with significantly less carbohydrate and lipid intake. In contrast, protein intake was higher in females in the MCR+HIW group than in the HIW group.

3.4. Body composition

As shown in **Fig. 2A**, although BMI in females and males continued to decrease after the start of intervention in both groups, the decrease was more rapid in the MCR+HIW group than in the HIW group with significant differences between the groups at the 8th and the 16th weeks in females.

This decrease in BMI was significantly correlated with total energy intake when the data in both groups were pooled (r = 0.267, P = 0.034; **Fig. 3A**) but this was not the case for energy expenditure by HIW (r = 0.110, P = 0.390; **Fig. 3B**).

3.5. Blood pressures

As shown in **Fig. 2A**, systolic and diastolic blood pressures in both groups showed nearly identical patterns to those for BMI after the start of intervention but with a significantly greater decrease in systolic blood pressure in the MCR+HIW group than in the HIW group only at the 8th week in females.

As shown in **Fig. 2B**, to investigate the effects of the combination of MCR and HIW in hypertensive subjects, subjects were divided into two subgroups: hypertensive (either systolic pressure ≥ 140 mmHg or diastolic pressure ≥ 90 mmHg) and normotensive according to their values at baseline. As shown in the figure, BMI and systolic blood pressure in the hypertensive subgroup of the MCR+HIW group decreased more rapidly than in the hypertensive HIW group.

3.6. Blood chemicals:

As shown in **Table 2**, LDL-C and TC concentrations in both the HIW and MCR+HIW groups decreased significantly after intervention (P = 0.001 and 0.017, respectively) and nearly returned to the normal range except in females in the MCR+HIW group, although this group was closer to the normal range than before intervention. Since HDL-C at baseline was much higher than 0.40 g/L, the standard value for Japanese (Matsuzawa, 2005) in both groups, it did not increase further after intervention. TG also decreased significantly in both groups after intervention (P = 0.047). Moreover, fGlc in both the HIW and MCR+HIW groups decreased significantly after intervention (P<0.001). However, we found that neither the effect of treatment nor the interaction of time and treatment were significant, suggesting that there was no significant difference in the decreases after intervention between groups.

3.7. VO_{2peak}

VO_{2peak} and HR_{max} increased significantly in all groups after intervention, except for HR_{max} in males, but the effect of treatment and the interaction of time and treatment were not significant in either group (**Table 3**).

3.8. Isometric knee forces

Isometric knee force did not increase significantly in either of the two groups for either gender (**Table 3**). Although total time of high-intensity walking during intervention tended to be shorter in the MCR+HIW group than in the HIW group (**Table 2**), there were no significant differences in isometric knee force between groups.

3.9. Total medical expenditure

Medical expenditure by the subjects for the 6 months from October 1, 2004 to March 31, 2005 before the start of the intervention was not significantly different between the HIW (17 subjects) and MCR+HIW (20 subjects) groups (**Table 6**). However, the expenditure for the 6 months from April 1 to September 30, 2005, during the time of intervention, was 53 617

312 yen lower in the MCR+HIW group than in the HIW group.

313

314 **4. Discussion**

315 In the present study, we found that a combined treatment with HIW training and MCR
316 for 16 weeks decreased BMI more than HIW training alone in middle-aged and older
317 subjects with mild obesity, and moreover, that the combined treatment decreased systolic
318 blood pressure more than HIW training alone in hypertensive subjects. Finally, we found
319 that the combined treatment decreased medical expenditure during intervention more than
320 HIW training alone.

321 Exercise has been broadly recommended as a countermeasure against
322 lifestyle-associated diseases and deteriorating physical fitness in young (Azegami et al.,
323 2006; Kondo et al., 2006) and older people (Ito et al., 2001; Tsuji et al., 2003; Hioki et al.,
324 2004; Okita et al., 2004; Hasegawa et al., 2005). Moreover, a higher intensity of aerobic
325 training (>50% VO_{2peak}) has been recommended in recent guidelines to increase VO_{2peak}
326 regardless of age (Armstrong et al., 2006). However, few regimens in the field provide
327 this increased intensity of aerobic exercise with the participation of walking regimens.

328 Recently, Nemoto and colleague suggested that HIW training increased VO_{2peak} and
329 knee muscle force, and decreased blood pressure more than moderate intensity of walking
330 in middle-aged and older people after 5 months of training (Nemoto et al., 2007). In the
331 present study, we confirmed that VO_{2peak} increased, blood pressure decreased, and blood
332 chemicals were improved regardless of group, although we failed to find any increase in
333 knee force. Regarding the mechanisms for increased VO_{2peak} after training in
334 middle-aged and older subjects, it has been suggested that this is due to enhance oxygen
335 extraction in skeletal muscle as well as increased cardiac stroke volume (Okazaki et al.,
336 2002, Nemoto et al., 2007). In addition to these factors, significant increases in HR_{max}
337 might also be involved in the increase in VO_{2peak} in this present study. Thus, HIW is a

useful training regimen to prevent lifestyle-associated diseases and deteriorating physical fitness.

According to the nutrition guidelines for Japanese aged 40 to 69 years issued by the government, energy intake for the grade II activity of walking or jogging every day is 1 950 kcal/day for females and 2 400 kcal/day for males, recommended protein intake is ≥ 50 g/day for females and ≥ 60 g/day for males, lipid % of total energy intake is < 25% for both genders, an carbohydrate % of total energy intake is <70% (Ministry of Health, Labor and Welfare, Japan, 2004). In the present study, as shown in **Table 5**, energy intake in the HIW group was 1 924 kcal/day for females and 2 311 kcal/day for males, protein intake was > 60g/day for both genders, lipid % of total energy intake was 27.1% in females and 27.3% in males, and carbohydrate % of total energy intake was 51.6% for females and 50.0% for males. Thus, the diets for subjects in the HIW group met the recommended nutritional guidelines for Japanese aged 40 to 69 years well, although the lipid % was slightly higher than recommended.

MCR treatment using meal replacement formula has been used for the treatment of obesity and significant effects have been found in previous studies (Okura et al, 2007; Nakano et al, 2008). In the present study, we asked subjects in the MCR+HIW group to continue their normal dietary habits including alcohol intake except for substitution of a meal replacement formula for lunch or dinner. As a result, energy intake in the HIW+MCR group was estimated to be 87% of that in the HIW group, which was attained by 18% and 17% reductions in carbohydrate and lipid intake, respectively. Based on these results, we believe that calorie restriction was appropriately mild in our experiment.

As shown in **Fig. 2A**, combined treatment with HIW exercise and MCR decreased BMI more than HIW training alone when the values were pooled from females and males;

-5.8% in the MCR+HIW group vs. -2.2% in the HIW group ($P<0.01$). Also, the treatment decreased systolic blood pressure more than HIW exercise alone at the 8th week in females; -7.0% in the MCR+HIW group vs. -4.0% in the HIW group. These results suggest a close association between BMI and systolic blood pressure.

To assess these data further, we compared the reductions in BMI and systolic blood pressure after training between hypertensive and normotensive subjects (**Fig. 2B**). As shown in the figure, we found that BMI and systolic blood pressure both decreased more in the MCR+HIW group than in the HIW group only in the hypertensive subjects, suggesting that a decrease in BMI evoked a reduction in systolic blood pressure, which could be accelerated by restricted energy intake. Indeed, it has been suggested that a reduction in blood pressure is associated with weight loss in hypertensive and overweight females (Kawamura et al., 1996).

In **Fig. 3**, we examined which was more effective in reducing BMI: restricted energy intake or increased energy expenditure with HIW. As shown in the figure, the reduction in BMI was significantly correlated with energy intake per day but not with total energy loss from HIW, suggesting that restricted energy intake was necessary to decrease BMI during HIW training. These results support the idea that CR or diet with exercise are essential for reduction of BMI in overweight people and are in agreement with previous studies (Svendsen et al., 1993; Okazaki et al., 2001; Colak and Ozcelik, 2004; Nicklas, 2004).

As shown in **Table 4**, total time of high-intensity walking tended to be shorter in the MCR+HIW group than in the HIW group with significance in females. Despite this, knee force was well maintained with no significant difference between groups in either gender. The detailed mechanisms of this remain unclear; however, previous studies have suggested that a combination of a high-protein diet and exercise enhanced an increase in muscle strength by exercise training (Wolfe, 2000; Layman et al., 2005). In the present study, we found that protein intake was higher than the recommended values (≥ 50 g/day (female) and ≥ 60 g/day (male)) (Ministry of Health, Labor and Welfare, Japan, 2004), which might significantly contribute to the maintenance of isometric knee force in the MCR+HIW group despite the shorter period of high-intensity walking training. Alternatively, the exercise which was carried out by the MCR+HIW group may have been sufficient to maintain muscle strength, or their weight loss may not have been enough to cause significant muscle mass loss in this period of training.

A prospective study in Japan indicated that medical expenditure was significantly lower in those walking for more than 1 h per day than in those walking for less than 1 h (Okita et al., 2004). In the present study, we found that the total medical expenditure for 6 months including the training period in the MCR+HIW group was significantly lower than that of the HIW group. The greater reduction in BMI and systolic blood pressure in the MCR+HIW group may be responsible for this reduced medical expenditure.

One limitation of the present study is that, no "no walking group" or "moderate-intensity continuous walking group" was used as a control. Therefore, the isolated effects of HIW or MCR on the measurements obtained in this study remain unknown. However, in our previous study (Nemoto et al., 2007), we found that isometric knee extension and flexion force and VO_{2peak} increased with marked reductions in systolic and diastolic blood pressures after HIW training for 5 months, similar to the results in the present study except for the change in knee force, while in contrast, these changes were minimal in a sedentary group and also in a moderate-intensity continuous walking group in our previous study. The reason why no increase was seen in knee force in the present study may be a result of the fact that knee force before training was 40-50% higher in the present cohort than in the previous study (Nemoto et al., 2007).

Based on these findings as a background, the present study was designed to examine the influence of MCR on improvements in physical fitness and indices of lifestyle-associated diseases by HIW training, and we found that VO_{2peak} increased in the MCR+HIW group in a similar manner to the HIW group while knee force remained unchanged in both groups, suggesting that MCR did not diminish the effects of HIW training. Also, we found that MCR accelerated the reduction in BMI by HIW training although it did not enhance the improvement in blood biochemical parameters. Thus, the HIW group can be regarded as a control group in the present study.

Another limitation in the present study is that a few married couples among the study subjects wanted to be in the same group and, a few others assigned to the HIW group wanted to join the MCR+HIW group, and thus minor reassignments were made. Therefore, strictly speaking, the subjects were not completely randomly divided into two groups. However, since there were no significant differences in training achievements between groups (**Table 4**), we are certain that there were no differences in their motivation for HIW training. Indeed, we found that total time for high-intensity walking tended to be rather attenuated in the MCR+HIW group.

Finally, since we determined energy intake in this study based on the diet diaries of study subjects, it is possible that these accounts are less precise than information directly determined by dietitians in hospitals. However, since subjects were interviewed by dietitians every 2 weeks and were well instructed on how to record their dietary intake, we feel that this is a reliable indicator of food intake during the intervention. Indeed, as shown in **Table 5**, we confirmed that the intake of energy, carbohydrates, and lipids was

434 significantly lower in the MCR+HIW group than in the HIW group.

435 In conclusion, HIW training improved the physical condition of middle-aged and
436 older overweight or mild-obese people, and combination with MCR at the beginning of
437 this exercise is more efficient than HIW training alone, especially for hypertensive
438 subjects. Moreover, we found that the MCR+HIW subjects also had lower medical care
439 expenditures than HIW subjects. Thus, this combination therapy has a greater effect on
440 reducing medical expenses than therapy with exercise alone. Further investigation using
441 variations on these unique diet and exercise treatments may further elucidate the most
442 effective, safest, and most sustainable methods for treating middle-aged and older people.

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Figure Legends

Figure 1: Study design. Overweight or obese subjects > 40 years old (BMI ≥ 23.6 kg/m^2) recruited from the Jukunen Taiiku Daigaku Program, were divided into HIW and MCR+HIW groups. After getting accustomed to HIW training for 2 weeks, subjects performed HIW training for at least 4 days/week, during which time their dietary intake was recorded for 3 consecutive days every 2 weeks. They visited a local community office every 2 weeks to have their data reviewed by trainers and dietitians, and also to have their body weight and blood pressure measured, and they were instructed how to perform HIW, if necessary. Subjects in the MCR+HIW group consumed a meal replacement formula with low carbohydrate and fat content and high protein content replacing either lunch or dinner every day for 16 weeks, and they were instructed to record their food intake in diaries for 3 consecutive days every 2 weeks and to submit it to a dietitian when they visited the local community office. Before and after the intervention, peak aerobic capacity, isometric knee force, and blood chemical concentrations were measured.

Figure 2: Percent change in BMI and blood pressure from baseline during intervention. Each point shows the mean ± SEM (open symbols, HIW group; closed symbols, MCR+HIW group). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. baseline. † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.001$ vs. at 8 weeks within the group. § $P < 0.05$, §§ $P < 0.01$, §§§ $P < 0.001$ vs. HIW group at the corresponding time. (**A**) Female HIW (n =17), MCR+HIW (n =30); male HIW (n =6), MCR+HIW (n =10); and total HIW (n =23), MCR+HIW (n =40). (**B**) Hypertensive HIW (n =5), MCR+HIW (n =15), and normal HIW (n =13), MCR+HIW (n =18).

Figure 3: Relationship between changes in BMI and energy intake per day (**A**) and

657 changes in BMI and total energy loss by HIW training during intervention (**B**). Each
658 point shows a value for each subject (open symbols for the HIW group (n = 23); closed
659 symbols for the MCR+HIW group (n = 40)). ΔBMI represents % change from baseline
660 calculated from Table 1. ΔBMI (y) was significantly correlated with energy intake/ day
661 (y); y = 0.002x -0.95, r = 0.267, P = 0.034, but not with total energy expenditure with
662 HIW training alone (P = 0.390).

Table 1 Physical characteristics and blood pressures before and after HIW training

	Female		Male		Total	
	HIW	MCR+HIW	HIW	MCR+HIW	HIW	MCR+HIW
Number	17	30	6	10	23	40
Age (year)	60.3 ± 1.6	60.5 ± 1.1	65.2 ± 1.8	65.9 ± 1.4	61.6 ± 1.3	61.9 ± 0.9
Height (cm)	152.5 ± 0.8	153.6 ± 1.0	165.1 ± 1.3	164.1 ± 1.6		
Body weight (kg)						
Baseline*	61.1 ± 1.8	61.5 ± 0.9	69.7 ± 1.6	72.1 ± 2.2		
Intervention	59.0 ± 1.8	57.8 ± 0.8	66.9 ± 1.7	68.0 ± 2.2		
BMI (kg/m^2)						
Baseline	26.3 ± 0.9	26.1 ± 0.3	25.6 ± 0.4	26.7 ± 0.6	26.1 ± 0.7	26.2 ± 0.3
Intervention	25.4 ± 0.9	24.5 ± 0.3	24.6 ± 0.4	25.2 ± 0.5	25.2 ± 0.6	24.7 ± 0.3
Blood pressures (mmHg)						
Systonic						
Baseline	127.0 ± 3.2	134.0 ± 3.0	131.8 ± 3.4	138.5 ± 6.6	128.1 ± 2.6	135.1 ± 2.7
Intervention	121.9 ± 3.7	120.7 ± 2.3	117.0 ± 6.0	125.5 ± 5.1	120.8 ± 3.2	121.9 ± 2.1
Diastolic						
Baseline	79.3 ± 2.2	79.9 ± 1.7	77.5 ± 3.9	81.0 ± 3.4	78.9 ± 1.9	80.2 ± 1.5
Intervention	72.3 ± 2.5	73.8 ± 1.7	65.5 ± 3.8	74.8 ± 4.0	70.8 ± 2.2	74.0 ± 1.6

Data are means ± SEM. *, There are no significant differences in any categories (baseline) between the HIW and MCR+HIW groups (Student's *t* test). Statistical analysis of BMI and blood pressures after the intervention term were shown in Fig. 2.

Table 2 Blood biochemical parameters before and after HIW training

	Female		Male		Total	
	HIW	MCR+HIW	HIW	MCR+HIW	HIW	MCR+HIW
Number	17	30	6	10	23	40
LDL-C (g/L)						
Baseline*	1.46 ± 0.07	1.48 ± 0.06	1.24 ± 0.08	1.40 ± 0.08	1.40 ± 0.06	1.46 ± 0.05
Intervention	1.33 ± 0.07	1.44 ± 0.06	1.12 ± 0.1	1.23 ± 0.08	1.27 ± 0.06	1.39 ± 0.05
P (time)**	0.012		0.009		0.001	
HDL-C (g/L)						
Baseline	0.68 ± 0.04	0.70 ± 0.02	0.50 ± 0.04	0.53 ± 0.02	0.63 ± 0.03	0.66 ± 0.02
Intervention	0.70 ± 0.04	0.71 ± 0.02	0.53 ± 0.03	0.59 ± 0.04	0.65 ± 0.03	0.68 ± 0.02
P (time)	NS		0.044		NS	
TC (g/L)						
Baseline	2.23 ± 0.08	2.29 ± 0.06	1.87 ± 0.06	2.06 ± 0.07	2.13 ± 0.07	2.23 ± 0.05
Intervention	2.12 ± 0.09	2.25 ± 0.06	1.78 ± 0.10	1.94 ± 0.08	2.03 ± 0.08	2.17 ± 0.06
P (time)	NS		0.034		0.017	
TG (g/L)						
Baseline	1.16 ± 0.15	1.01 ± 0.09	1.38 ± 0.23	1.43 ± 0.26	1.21 ± 0.13	1.12 ± 0.09
Intervention	1.00 ± 0.09	0.94 ± 0.06	1.18 ± 0.11	1.13 ± 0.05	1.05 ± 0.08	0.99 ± 0.06
P (time)	NS		NS		0.047	
fGlc (g/L)						
Baseline	1.05 ± 0.02	1.01 ± 0.02	1.22 ± 0.08	1.09 ± 0.07	1.09 ± 0.03	1.03 ± 0.02
Intervention	1.00 ± 0.02	0.97 ± 0.02	1.09 ± 0.07	0.98 ± 0.05	1.02 ± 0.02	0.97 ± 0.02
P (time)	< 0.001		< 0.001		< 0.001	

Data are means ± SEM. *, There are no significant differences in any categories (baseline) between the HIW and MCR+HIW groups (Student's *t* test). **, After the intervention, effects on treatment and on interaction of time and treatment were not significantly different between the HIW and MCR+HIW groups, but some effects of time were significantly different (2-way ANOVA for repeated measures). NS, not significant.

Table 3 Physical fitness before and after HIW training

	Female		Male		Total	
	HIW	MCR+HIW	HIW	MCR+HIW	HIW	MCR+HIW
Number	17	30	6	10	23	40
Isometric knee forces (N)						
Extension						
Baseline*	418.5 ± 21.2	443.9 ± 21.5	525.3 ± 75.6	645.4 ± 65.2		
Intervention	421.9 ± 25.4	436.5 ± 20.1	509.0 ± 78.3	618.6 ± 60.0		
P (time)**	NS		NS			
Flexion						
Baseline	214.9 ± 9.1	221.8 ± 7.9	263.7 ± 22.7	290.7 ± 20.4		
Intervention	221.2 ± 14.0	238.7 ± 8.6	281.0 ± 28.9	301.8 ± 28.9		
P (time)	NS		NS			
HR_{max} (beat/min)						
Baseline	129.2 ± 2.8	135.0 ± 3.0	130.1 ± 8.6	128.1 ± 6.4	129.4 ± 2.8	133.3 ± 2.8
Intervention	142.0 ± 3.5	146.0 ± 3.0	135.6 ± 10.9	136.2 ± 3.7	140.7 ± 3.4	143.4 ± 2.5
P (time)	< 0.001		NS		< 0.001	
VO_{2peak} (mL/min/kg)						
Baseline	19.9 ± 0.6	22.0 ± 0.8	21.5 ± 2.1	20.0 ± 1.3	20.2 ± 0.6	21.4 ± 0.7
Intervention	26.9 ± 1.4	28.2 ± 1.3	24.7 ± 3.8	25.2 ± 0.7	26.5 ± 1.3	27.4 ± 1.0
P (time)	< 0.001		< 0.001		< 0.001	

Data are means ± SEM. *, There are no significant differences in any categories (baseline) between the HIW and MCR+HIW groups (Student's *t* test). **, After intervention, effects on treatment and on interaction of time and treatment were not significantly different between the HIW and MCR+HIW groups, but some effects of time were significantly different (2-way ANOVA for repeated measures). NS, not significant.

Table 4 HIW training achievement

	Female		Male		Total	
	HIW	MCR+HIW	HIW	MCR+HIW	HIW	MCR+HIW
Number	17	30	6	10	23	40
Walking time and energy expenditure per day						
Low-intensity (min)	16.2 ± 2.7	29.7 ± 6.7	27.8 ± 12.2	24.5 ± 4.3	19.3 ± 3.7	28.4 ± 5.1
*P**	NS		NS		NS	
High-ntensity (min)	30.7 ± 3.0	21.6 ± 1.5	30.8 ± 5.7	28.8 ± 4.2	30.7 ± 2.6	23.4 ± 1.6
P	0.004		NS		0.014	
Sum (min)	47.0 ± 4.4	51.3 ± 6.3	58.6 ± 8.7	53.3 ± 5.0	50.0 ± 4.0	51.8 ± 4.9
P	NS		NS		NS	
Energy (kcal)	164.3 ± 17.4	157.6 ± 10.9	213.7 ± 23.9	210.5 ± 28.5	177.1 ± 14.8	170.8 ± 11.2
P	NS		NS		NS	
Total walking time and total energy expenditure						
Low-intensity (h)	24.5 ± 4.5	39.9 ± 6.9	57.7 ± 30.6	33.8 ± 6.7	33.2 ± 8.7	38.4 ± 5.4
P	NS		NS		NS	
High-ntensity (h)	48.1 ± 7.3	32.0 ± 3.1	52.7 ± 8.1	41.2 ± 8.1	49.3 ± 5.7	34.3 ± 3.1
P	0.023		NS		0.014	
Sum (h)	72.7 ± 10.4	71.9 ± 7.9	110.4 ± 26.1	75.0 ± 9.9	82.5 ± 10.5	72.7 ± 6.3
P	NS		NS		NS	
Energy ($x10^3$ kcal)	15.37 ± 2.42	14.21 ± 1.64	23.76 ± 4.18	17.41 ± 2.65	17.56 ± 2.19	15.01 ± 1.40
P	NS		NS		NS	

Data are means ± SEM. *, The significant differences between the HIW and MCR+HIW groups were analyzed with the use of Student's *t* test. NS, not significant.

Table 5 Energy intake and major nutrients during the intervention

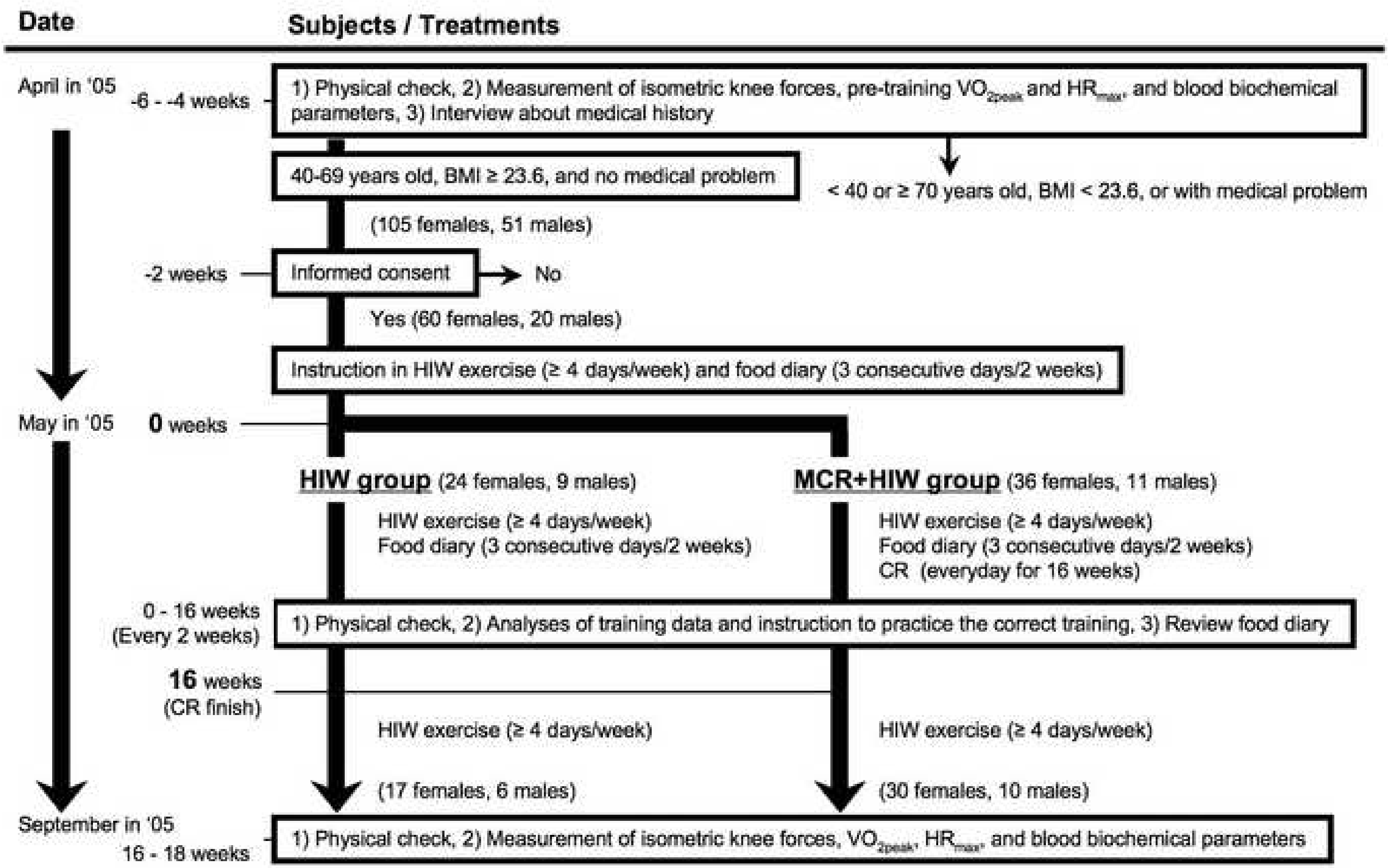
	Female						Male					
	HIW			MCR+HIW			HIW			MCR+HIW		
Number	17			30			6			10		
Intake energy (kcal/day)	1 924	±	64.9	1 675	±	38.3 (87.1%)*	2 311	±	227.8	2 020	±	57.1 (87.4%)
*P***	0.001						NS					
Nutrients (g/day)												
Carbohydrates	248	±	2.6	203	±	2.0	289	±	6.8	232	±	3.1
P	< 0.001						< 0.001					
Proteins	78	±	0.9	81	±	0.6	97	±	2.3	93	±	1.0
P	0.004						NS					
Lipids	58	±	1.0	48	±	0.6	70	±	2.1	53	±	1.1
P	< 0.001						< 0.001					

Data are means ± SEM. *, Percentages of energy intake on MCR+HIW group showed the % ratios versus that on HIW group for each gender. **, The significant differences between the HIW and MCR+HIW groups were analyzed with the use of Student's *t* test. NS, not significant.

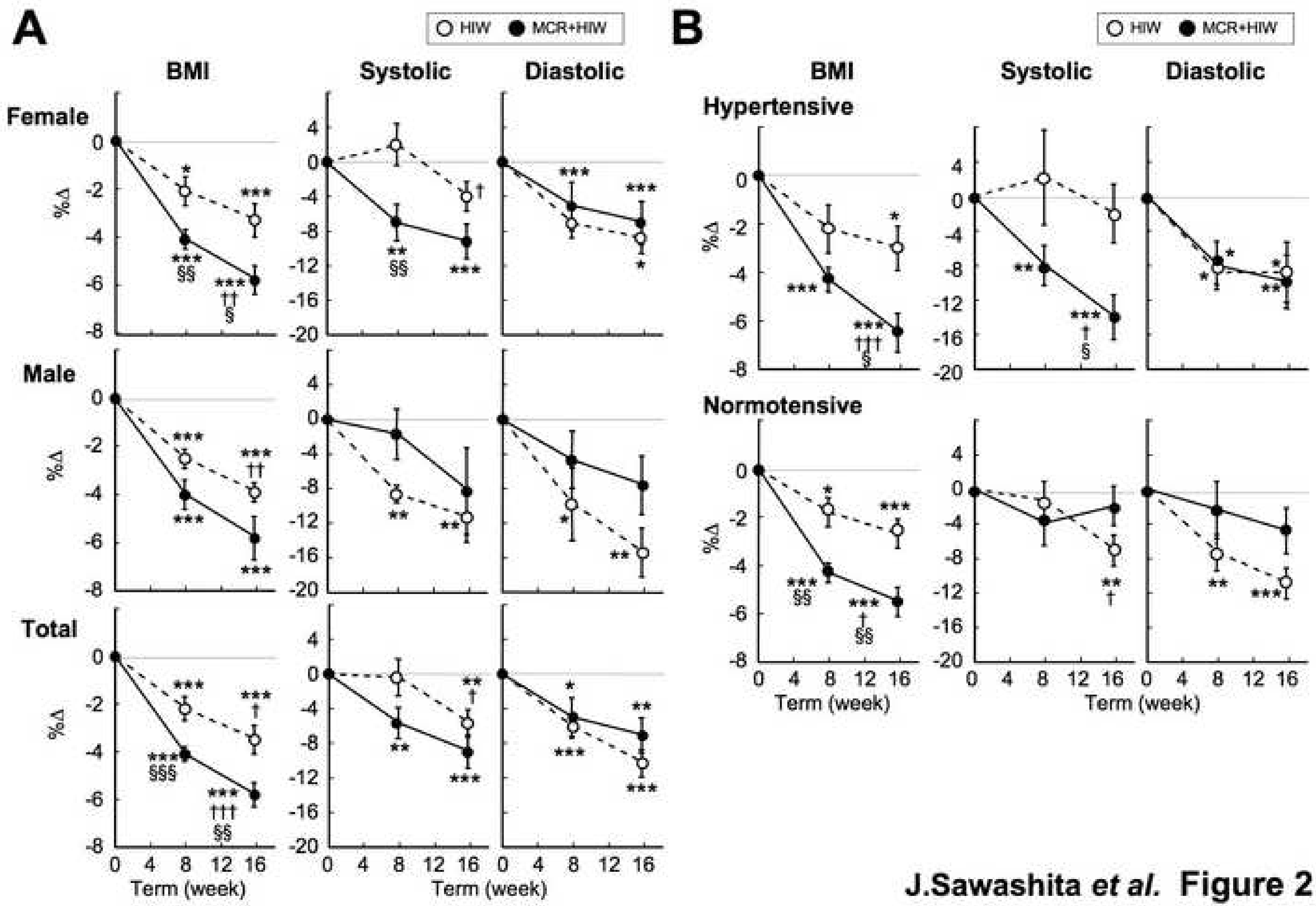
Table 6 Medical expenditure for individuals

	HIW			MCR+HIW		
Number*	17			20		
Expenditure (yen/6 months)						
Baseline (Oct '04- Mar '05)	68 028	±	13 138	48 336	±	10 793
*P***	NS					
Intervention (Apr - Sep '05)	90 554	±	21 384	36 937	±	8 931
P	0.03					

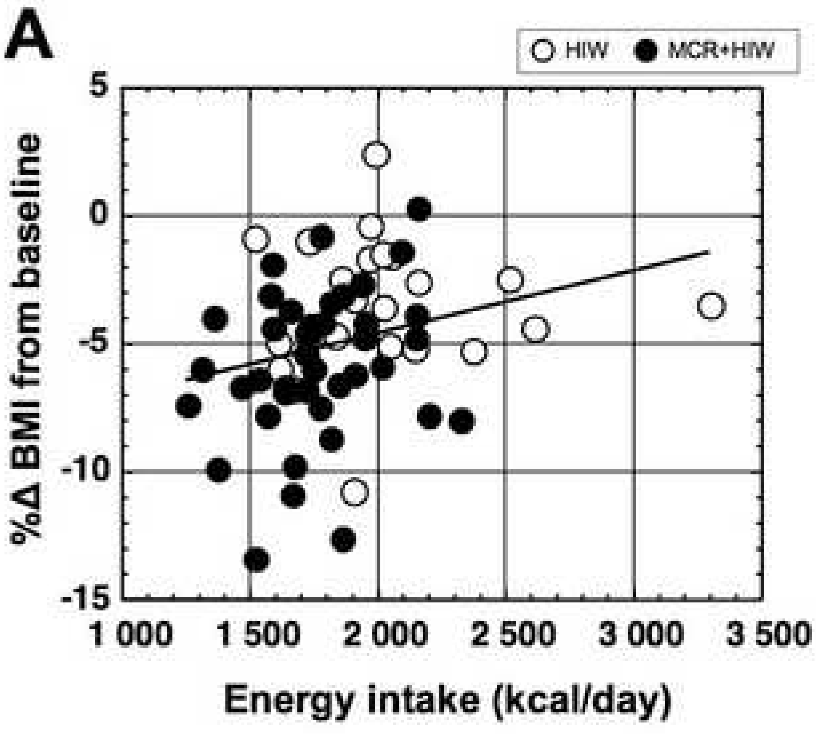
*, Number; members of the national health insurance system. Data are means ± SEM. **, The differences between the HIW and MCR+HIW groups were analyzed with the use of Student's *t* test. NS, not significant.



J.Sawashita *et al.* Figure 1



J.Sawashita *et al.* Figure 2



J.Sawashita *et al.* Figure 3