早稲田大学審査学位論文

博士（スポーツ科学）

EFFECTS OF PHYSICAL ACTIVITY PATTERNS AND AEROBIC CAPACITY ON FAT UTILIZATION OVER A WHOLE DAY

身体活動パターンと有酸素性能力が 1 日の脂質利用量に及ぼす影響

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Introduction

The importance of the primary prevention of obesity

The increase in the prevalence of obesity has become a global epidemic in many industrialized and developing countries. Obesity is a strong risk factor for the development of cardiovascular disease, type 2 diabetes mellitus, and certain types of cancer. As reported by Jakicic et al. (30), only approximately 27% of individuals participating in a behavioral weight-loss intervention achieved a 10% weight loss at 24 months, with 49% of individuals achieving at least 5% weight loss. This suggests that it is difficult to achieve long-term weight loss, and that it is important to focus on prevention of obesity.

A cause of obesity: The difficulty of measuring weight gain

Obesity develops as a consequence of long-term positive energy balance (EB), reflecting an imbalance between energy intake (EI) and energy expenditure (EE), or a habitual EI excess. Hill et al. (28) has reported that the average daily excess of total EI (TEI) over total EE (TEE) in 90% of the population in America is 50 kcal/day or less, based on data from the National Health and Nutrition Examination Survey (NHANES) and the Coronary Artery Risk Development in Young Adults (CARDIA) study. However, previous studies have indicated the difficulty associated with detecting the energy surplus (excess EI) in free-living conditions, particularly if the actual energy surplus continuously accumulates at less than 50 kcal daily. To

put this into perspective, precision of the doubly labeled water (DLW) technique, the "gold standard" method for the measurement of TEE, can vary 5-8%, or by about 200 kcal/day over a 1-2 week period. All other methods for the evaluation of TEE have lower accuracy and precision than the DLW method. Thus, energy flux can only be quantified with a maximum accuracy in the order of 100 kcal. In addition, the DLW technique cannot detect a change in magnitude of daily EE. Therefore, successive monitoring and control of weight gain using current technologies is difficult, and consideration should be given to strategies designed to promote the prevention of weight gain.

Cases of overeating

The cause of excess EI and its individual variability typically involves cognitive and/or physiological problems. Binge eating at parties during holidays is an example of a more easily recognizable type of overeating which may be associated with “behavioral overeating.” Alternatively, “physiological overeating” is considered to be involuntary and instinctive overeating, possibly due to the desire to survive. The causes of excess EI are complex in relation to both the behavioral and physiological origins for overeating.

Overeating due to dysfunctional energy balance (EB) regulation

In all probability, the disturbance of EB regulation or homeostasis is one of the key reasons for overeating. Humans have numerous physiological functions that contribute to strict

maintenance of energy homeostasis. However, if EB regulation is dysfunctional, it may become one of the causes of overeating. Several previous studies have shown that obese individuals differ from those who are nonobese in their precision of energy regulation in relation to ingested calories (37, 55, 65); however, complete agreement among previous study results is lacking (11, 54) and there is no evidence that individuals with less ability to regulate EB succumb to subsequent weight gain.

Interestingly, many studies have indicated that habitual exercise, or a higher physical activity level (PAL), helps weight maintenance via improvements related to adequate food demand (7, 41, 42, 68, 69). This hypothesis was first suggested by Mayer et al. (41, 42). Maintenance of a higher PAL should make an acute negative EB easier to achieve as the orexigenic response should take longer to come into effect. In addition, metabolic functions for the maintenance of EB are likely to be more sensitive during negative EB than during positive EB (78). Thus, humans may more easily consume excess energy under conditions of physical inactivity as many studies have shown.

Furthermore, the concept of better coupling of EI to EE in highly active individuals has been supported using the preload test. Several papers have demonstrated that habitual exercise, or higher PA, improves acute appetite control and facilitates the balance of postprandial energy to a subsequent meal (35, 38, 40, 72). Recently, I have paid attention to the

effect of habitual PA patterns on appetite regulation, and had a presentation that prolonged sedentary behavior has been associated with a reduced ability to promptly regulate EI to compensation for previous EI (1). However, further studies are needed to confirm this postulated cause and effect relationship and to clarify how sedentary activity affects appetite regulation.

Overeating due to behavior and physiological function can covertly sabotage EB regulation

The traditional concept of the EB equation, which describes weight gain as positive energy imbalance, can be replaced by a series of macronutrient balance equations in which body fat stores are viewed specifically as an imbalance of fat (15). The notion proposed by Flatt, is that interconversion between the macronutrients is negligible, and oxidative priority operates in inverse proportion to the size of available stores for each macronutrient. Alcohol is most readily oxidized because it cannot be stored. Oxidation of carbohydrate and protein are also under tight auto-regulatory feedback control. Considering that the body's glycogen storage capacity is limited to 200-800 g, an average daily carbohydrate intake of 350 g corresponds to 44-175% of glycogen storage capacity. In contrast, there is no acute feedback between fat intake and fat oxidation, because the largest energy stores in the body are fat stores in adipose tissue. In other words, fat oxidation can bridge the gap between TEE and the amounts of alcohol, protein,

carbohydrate, and fat consumed. Thus, dietary fat oxidation is poorly correlated with daily variations in fat consumption.

This mechanism may play an important role in overeating under free-living conditions. In situations of free-living, there are added complexities when it comes to diet composition, energy content, PA, and EB, all of which vary considerably from meal-to-meal and from day-to-day. In particular, attention is often focused on switching dietary macronutrient composition from high-carbohydrate (HC) to high-fat (HF). This situation can lead to excess carbohydrate oxidation (negative carbohydrate balance). The dilemma here is that a negative carbohydrate balance is likely to reduce glycogen stores and subsequently induce feelings of hunger. Several studies have recently supported the link between negative carbohydrate balance and increased food intake under carefully controlled situations (18, 39, 51). Moreover, several prospective observational studies suggest that lower fat oxidation capacity predicts both weight and fat gain over a year or longer (12, 13). Conversely, an individual with a high fat oxidation capacity can potentially attenuate a negative carbohydrate balance.

To summarize, in relation to the recent studies discussed, there is an awareness of the misleading of appetite control that occurs, even when EI and EE are balanced. An explanation for this phenomenon, at least in part, suggests that it is attributable to modern eating habits, particularly as a result of switching from an HC meal to an HF meal. This eating habit is likely

to promote weight gain due to a surplus of ingested fat. Moreover, because binge eating at a party or a large reduction in the level of PA during holidays can result in a surplus of fat, this behavior may promote a subsequent weight gain.

How do we prevent the predisposition for overeating that is sabotaging EB regulation due to the surplus of ingested fat?

An effort of this study was made to try to determine the behavior and characteristics that drive individuals to ingest a surplus of dietary fat. In particular, attention was focused on incorporating PA into the first study and aerobic capacity (AC) into the second study because PA is a major determinant of dietary fat oxidation, and AC can be one of the most modifiable parameters regulated by body physiological functions to maximize substrate oxidation capacity.

Methods

Subjects

The study protocol was approved by the Ethical Committee of the National Institute of Health and Nutrition (NIHN) in Japan. Ten Japanese non-obese healthy young men participated and gave written informed consent for this study. However, only nine participants were used in the current analysis, because one was regarded to have dyslipidemia; the blood triacylglycerol (TG) and low-density lipoprotein cholesterol (LDL-C) levels of the excluded participant were above 150 mg/dL and 140 mg/dL, respectively, under fasted conditions on day 2 in both trials. Participants were non-smokers, non-shift worker adults and had no chronic diseases affecting metabolism or PA such as diabetes, metabolic disease, or digestive disease. They did not use any medicines or supplements. In addition, when participants were recruited, men who performed specific exercises on a regular basis that would prevent them from wearing an accelerometer (e.g., swimming) were excluded.

Experimental design

After completing medical history screening including food allergies, anthropometric measurements and a habitual PA questionnaire (International Physical Activity Questionnaire: IPAQ) were completed, and maximum oxygen uptake was measured in the morning under fasted conditions. Body fat mass (FM) and fat-free mass (FFM) were measured using

dual-energy x-ray absorptiometry (Hologic QDR-4500; Hologic, Inc., Bedford, USA). Participants completed two trials, with an interval of 10 days to 3 weeks. Each participant wore a tri-axial accelerometer (Active style Pro HJA-350IT; Omron Healthcare, Kyoto, Japan) during all measurement periods after trial commencement (about one month).

This was a randomized study using a crossover design. Each participant performed two PA trials (continuous and intermittent exercise), each involving a 39-hour session (2 nights and 3 days) in a respiratory chamber (Figure 1). During the day while participants stayed in the room, intentional vigorous PA such as exercise was restricted. Participants consumed all meals provided until 1330 h and drank water freely. Participants arrived at the NIHN in the evening at 1730 h, were weighed lightly clad, put on the accelerometer, were fitted with an electrode for measuring heart rate, and entered the calorimeter at 1850 h. They consumed the HC meal at 1930 h and were permitted to sleep at 2300 h. The HF experiments commenced at 0800 h on day 2 after an equilibration period and finished at 1005 h on day 3. While in the calorimeter, participants adhered to an identical fixed schedule of eating meals, exercising, and sleeping. Minimum sleeping metabolic rate (SMR) was recorded overnight and calculated as minimum EE over 3 hours on each of the 2 consecutive nights. Other metabolic parameters while sleeping were analyzed from 2305 h to 0700 h on each of the 2 consecutive nights. Participants temporarily left the chamber from 0715 h until 0730 h on the 2 consecutive mornings to provide

a blood sample.

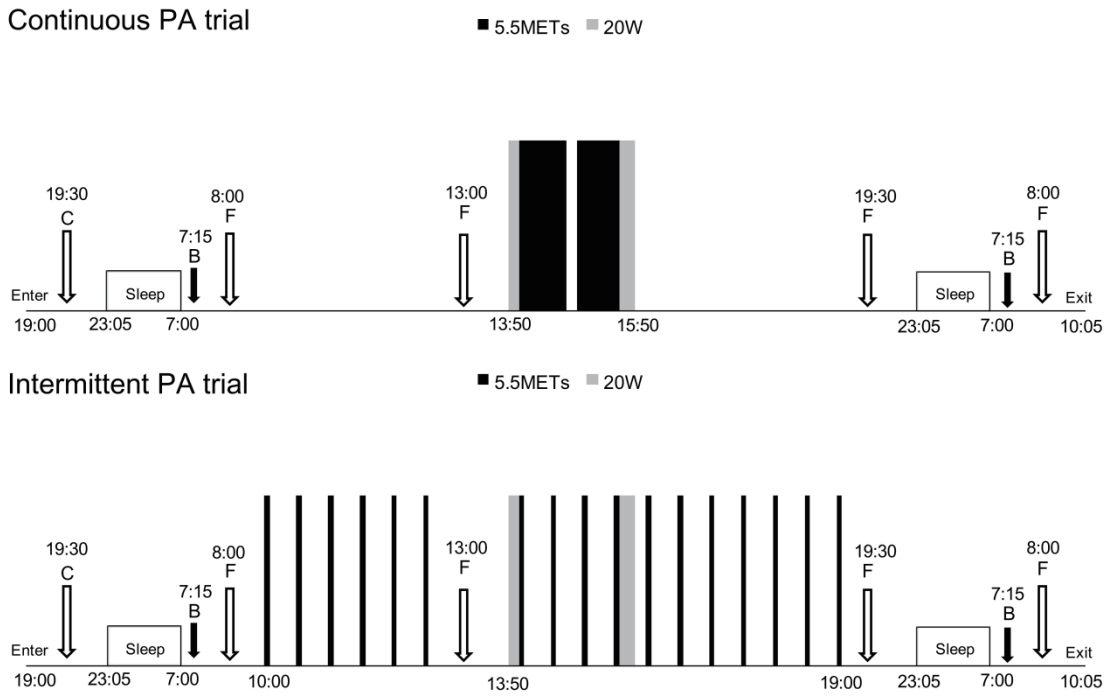

Figure 1. Protocols in the continuous PA and the intermittent PA trials in the chamber. ***Black*** **bar, 5.5 METs cycling;** ***gray*** **bar, 20-W cycling;** ***open*** **arrows, meal eating; filled arrows, blood sampling. C, carbohydrate meal; F, fat meal; B, blood sampling.**

Dietary treatments

For 2 days before entering the chamber, participants consumed a provided HC weight maintenance diet (15% of kcal from protein, 15% from fat, and 70% from carbohydrate). Energy requirements were calculated individually as estimated by basal metabolic rate (BMR) × habitual PAL. BMR was estimated from age, sex, height, and body weight using Ganpule's equation (20), derived from Japanese adults at the NIHN. Habitual PAL was estimated using IPAQ criteria, and 1.7 or 1.9 was applied, based on a report on the relationship between the IPAQ criteria and PAL evaluated by the DLW method for Japanese adults (29). Energy intake (EI) of the participants was either 2000, 2500, or 3000 kcal/d depending on their estimated energy requirements. In addition, participants were asked to spend some time as similar as possible during the 2 days before entering the chamber for each trial.

Participants consumed the HF meals (15% of kcal from protein, 50% from fat, 35% from carbohydrate) starting from breakfast on day 2. They consumed the same meal 4 times (breakfast, lunch, dinner, and breakfast) to avoid interactions of differences in food with time. Energy requirements in the chamber were calculated individually as estimated BMR × 1.6 (PAL of 1.6). The EI of a total of 3 meals (breakfast, lunch, and dinner) for each participant was either 2200, 2400, 2600, or 2800 kcal/d depending on their estimated energy requirements. Fatty acid profiles of all meals consumed in the chamber were held constant with equal proportions of

saturated, mono-unsaturated, and poly-unsaturated fatty acids (1.4: 1.0: 0.5). All diets were commercial products (processed foods) with known nutritional status and were provided by a dietitian.

Exercise

The participants performed a total of 85 minutes of exercise using a static cycling ergometer (Aerobike 75XL ii; Combi Wellness Corporation, Tokyo, Japan) at workload of 5.5 metabolic equivalents (METs) determined for each individual. In the continuous PA trial, participants started exercise at 1350 h and finished at 1550 h. They performed a 10 minute warming up, 45 minutes of exercise followed by a 10 minute break, then 40 more minutes of exercise, and a 15 minute cooling down. In the intermittent PA trial, the participants started exercise at 1000 h and finished at 1905 h. They performed 5 minutes of exercise every 30 minutes for a total of 17 bouts. They performed an identical 10 minute warming up and 15 minute cooling down at the same time as in the continuous PA trial. The workload for warming up and cooling down was 20 W.

The workload of 5.5 METs was calculated by regression of the 20, 60, and 90 W points while measuring maximum oxygen uptake. Maximum oxygen uptake was measured by increasing the load every 3 minutes until exhaustion. Expired gas was sampled into Douglas bags, and VO2 was obtained from oxygen and carbon dioxide concentrations measured using a

mass spectrometer for respiration (ARCO-2000; ARCOSYSTEM, Kashiwa, Japan) and gas volume measured using a dry process gas flow meter (DC-1; Shinagawa, Tokyo, Japan) for the last 30 seconds of every stage. The number of revolutions for all ergometer trials was kept at 60 r/min to keep energy efficiency constant. VO2 was considered “peak” if two of the following criteria were met: 1) measured HRmax ≥ age-predicted HRmax - 10 beats/min; 2) VO2 increased by < 100 mL/min during a trial; 3) RERmax was ≥ 1.10; and/or 4) Borg Scalemax was ≥ 19.

Indirect calorimetry

The present study used 2 open-circuit human calorimeters at the NIHN to measure oxygen consumption and carbon dioxide production. Details of the human calorimeter were previously reported (20, 46). The accuracy of the chambers in measurement of EE as determined by an alcohol combustion test was 99.2 ± 0.7 (Mean ± SD) over 6 h and 99.2 ± 3.0 (Mean ± SD) over 30 minutes. The rooms were maintained at a temperature of 25 degrees centigrade, humidity of 55%, and a ventilation rate of 60 L/min. Ventilation rate was measured every 12 seconds using a pneumotachometer. Oxygen and carbon dioxide concentrations were also measured every 12 seconds using a mass spectrometer (AR-2000; Arco System, Kashiwa, Japan). EE was calculated from VO2 and VCO2 using Weir’s equation (77). Respiratory exchange ratio (RER) was defined as VCO2/VO2. Fat and carbohydrate oxidation were

calculated from VO2 and VCO2, and protein oxidation using Jequier's equation (32). Protein intake was substituted for protein oxidation.

Physical activity evaluation using a tri-axial accelerometer

Participants wore the tri-axial accelerometer on the waist until all experiments were completed. The accelerometer was developed especially for evaluating relatively low intensity PA and non-locomotive or household PA (49, 50). Data were recorded in 10 second epoch length. Cycling PA measured by accelerometry while using the cycling ergometer in the chamber was converted to an acceleration value using Ohkawara's equation (49). Non-wearing time while in the chamber was assigned a value of 0.9 METs based on Ohkawara's paper. Continuity of sedentary behavior (METs $\leq$ 1.5) in the chamber was scored using three cutoff points (3 or more, 5 or more, or 10 or more consecutive minutes), and analyzed using Microsoft Excel (2007; Microsoft Japan, Tokyo, Japan).

Blood sampling

Blood samples for each participant were obtained under fasting condition at 0715 h outside the chamber on 2 consecutive mornings to identify metabolically abnormal subjects and to confirm whether there was an interaction with lipid metabolism between trials (lipid oxidation subsides during the night). Blood samples collected in pre-chilled tubes that contained a serum separating medium were centrifuged for 20 minutes at 3000 r/min 30 minutes after

drawing blood, after which serum was immediately stored in a refrigerator. Blood samples collected in pre-chilled tubes that contained EDTA-2Na were centrifuged for 20 minutes at 3000 r/min, after which plasma was immediately stored in a freezer. Blood samples collected in pre-chilled tubes that contained EDTA-2Na and NaF were stored immediately in a refrigerator. Plasma concentrations of glucose, insulin, TG, nonesterified fatty acids (NEFA), high-density lipoprotein cholesterol (HDL-C), LDL-C, and norepinephrine were analyzed at Mitsubishi Chemical Medience Corporation.

Statistical analysis

Data are presented as means ± standard deviation (SD). 23-h RER from 0800 h on day 2 to 0700 h on day 3 was analyzed as the main outcome of this study. Descriptive statistics were calculated, and Student's paired t test, repeated measures analysis of variance, and Pearson's (partial) correlations were performed using SPSS (18.0; IBM SPSS, Tokyo, Japan). Student's paired t test was used to assess differences between the trials. Since RER during sleeping time on day 1 was significantly different between trials, and because sleeping RER was significantly correlated with 23-h RER, it was analyzed as a covariate. Multiple linear regression analysis was used to adjust for covariance. Biochemical data were statistically analyzed using repeated measures analysis of variance. The mean TEE and energy balance were significantly different between trials. However, because neither TEE nor energy balance was significantly correlated

with 23-h RER, these were not used as co-variates. Physical activity data were assessed for normality using a Kolmogorov-Smirnov test and kurtosis and skewness were determined; however all variables were normally distributed. Results were considered significant at $p < 0.05$.

Study1. Effects of physical activity patterns on fat utilization

INTRODUCTION

Prevalence of both obesity and obesity-related diseases have been rising in most developed countries (14). Physical activity (PA) is often recommended as a strategy for obesity prevention. The International Association for the Study of Obesity (IASO) has adopted a consensus statement stating that a PA level (PAL) of 1.7, or moderate intensity PA for 45 to 60 minutes per day, is recommended to prevent weight and fat gain in adults (57). However, a recent systematic review and another report indicated that weight changes cannot be fully explained by lower PA (80) or decreases in PA over time (79). Westerterp and Speakman (79), interestingly, reported that PAL in adults evaluated by the doubly-labeled water (DLW) method has not decreased since the 1980s. Therefore, it is possible that other factors related to PA, but independent of PAL, may influence weight and fat gain in adults.

Previous studies have reported that higher PAL induces greater fat utilization when switching from an HC to an HF meal (8, 23, 58, 63) . These studies showed results consistent with the IASO consensus statement. However, it has not been investigated whether PA patterns influence fat utilization during switching from an HC to an HF meal independent of PAL. In general, moderate intensity PA, and in particular prolonged moderate intensity exercise lasting for 10 minutes or more, is widely believed to be one of the easiest ways to utilize PA to increase

EE while burning a large proportion of energy from fat. In fact, most previous studies have investigated the effects of continuity of exercise with a duration over 10 minutes (47). However, increased accumulated intermittent PA and intense non-locomotive PA are effective alternatives to prolonged PA, and both increase EE and fat utilization. Troiano et al. (70) suggested that moderate to vigorous PA (MVPA) carried out for 10 or more minutes accounted for only one-third of total time spent on MVPA as measured using an accelerometer. Interestingly, it was shown that the amount or frequency of intermittent PA ("breaks in sedentary") may play a role in obesity-related outcomes (27), independent of total time spent on MVPA or sedentary behavior. Thus, although intermittent PA carried out for less than 10 minutes may be an important factor for PAL and obesity-related outcomes independent of MVPA, there is no evidence on whether intermittent PA influences fat utilization or subsequent weight or fat gain.

Therefore, the aim of the present study was to determine whether continuous and intermittent PA differentially influenced fat utilization over the course of a whole-day. We measured both continuous and intermittent PA using a human calorimeter over one day with an HF meal.

RESULTS

Subject characteristics.

Table 1 shows the characteristics of the nine participants. Participants were weight stable (within 1.5 kg) throughout the study period. Average total EE for two days before entering the chamber, measured using an accelerometer was not significantly different between the continuous PA trial and the intermittent PA trial with 2450 ± 225 kcal, 2522 ± 374 kcal, respectively. The same diets were provided between trials and all were consumed before entering the chamber. The mean total energy intake was 2660 ± 225 kcal in both trials, and mean macronutrient composition was 15.3 ± 0.0% protein, 15.8 ± 0.2% fat, and 68.9 ± 0.2% carbohydrate in both trials.

Table 1. Physical characteristics.

	Mean ± SD
Age (yr)	22.2 ± 0.6
Height (cm)	170.6 ± 3.9
Weight (kg)	63.03 ± 4.97
Body fat (%)	13.1 ± 2.8
VO2peak (ml· kg^{-1}· min^{-1})	46.3 ± 6.3
Percentage of VO2peak at 5.5 METs (%)	42.4 ± 6.4

EI, EE, and substrate oxidation under HC conditions in the chamber.

Average EE and RER for each segment are shown in Tables 2 and 3. On the first night, EE and RER were not significantly different between trials except for sleeping time RER. During sleep on day 1, RER in the intermittent PA trial was significantly lower than in the continuous PA trial ($p = 0.01$).

Table 2. EE/metabolic rate in each segment and low-intensity PA in the chamber

	Continuous	Intermittent	p value
Energy expenditure/metabolic rate			
1st day			
Post-dinner (1930-2300) (kcal· min^{-1})	1.488 ± 0.129	1.481 ± 0.090	NS
Sleeping (2305-0700) (kcal· min^{-1})	1.056 ± 0.088	1.036 ± 0.085	NS
Minimum Sleeping (kcal· min^{-1})	0.972 ± 0.088	0.969 ± 0.083	NS
2nd day			
23 h (0800-0700) (kcal)	2373 ± 198	2456 ± 155	*p = 0.01*
Post-breakfast(0800-0955) (kcal· min^{-1}, N=8)	1.595 ± 0.116	1.621 ± 0.075	NS
Cycling period (1000-1930) (kcal· min^{-1})	2.346 ± 0.225	2.458 ± 0.175	*p < 0.01*
Post-dinner (1930-2300) (kcal· min^{-1})	1.567 ± 0.112	1.623 ± 0.098	*p = 0.058*
Sleeping (2305-0700) (kcal· min^{-1})	1.064 ± 0.077	1.071 ± 0.075	NS
Minimum Sleeping (kcal· min^{-1})	0.995 ± 0.085	0.993 ± 0.074	NS
3rd day			
Post-breakfast (0800-0955) (kcal· min^{-1}, N=8)	1.644 ± 0.157	1.674 ± 0.105	NS
Physical activity with METs ≤ 1.5 (0800-2300)			
Total minutes (min)	671.4 ± 55.8	640.6 ± 54.4	*p < 0.05*
Consective minutes with METs ≤ 1.5 (min)			
3 or more consecutive minutes with METs ≤ 1.5	480.1 ± 130.4	393.9 ± 121.1	*p < 0.05*
5 or more consecutive minutes with METs ≤ 1.5	376.2 ± 143.1	288.1 ± 128.8	*p < 0.05*
10 or more consecutive minutes with METs ≤ 1.5	197.0 ± 101.6	110.6 ± 80.4	*p < 0.01*

Value are mean ± SD. Metabolic rate of post-breakfast were analyzed in eight males. PA with METs ≤ 1.5 was evaluated by using accelerometer. NS, nonsignificant.

Table 3. REE in each segment.

	Continuous	Intermittent	p value
Respiratory exchange ratio			
1st day			
Post-dinner (1930-2300)	0.886 ± 0.039	0.887 ± 0.029	NS
Sleeping (2305-0700)	0.860 ± 0.034	0.843 ± 0.031	*p = 0.01*
2nd day			
23h (0800-0700)	0.847 ± 0.015	0.833 ± 0.015	*p < 0.001*
Post-breakfast (0800-0955) (N=8)	0.892 ± 0.029	0.888 ± 0.029	NS
Cycling period (1000-1930)	0.869 ± 0.017	0.852 ± 0.020	*p = 0.001*
Post-dinner (1930-2300)	0.808 ± 0.012	0.788 ± 0.014	*p < 0.001*
Sleeping (2305-0700)	0.800 ± 0.014	0.791 ± 0.007	*p = 0.01*
3rd day			
Post-breakfast (0800-0955) (N=8)	0.835 ± 0.014	0.828 ± 0.017	NS

Value are mean ± SD. Metabolic rate of post-breakfast were analyzed in eight males. NS, nonsignificant.

EI, EE, and substrate oxidation under HF conditions in the chamber.

On day 2 in the chamber, mean total energy intake was 2413 ± 132 kcal in both trials, and macronutrient composition was 16.4 ± 0.3% kcal from protein, 49.0 ± 0.7% from fat, and 34.6 ± 0.5% from carbohydrate in both trials. Table 2 shows energy expenditure and metabolic rate values during each segment in the chamber. The mean total energy expenditure in the intermittent PA trial (2456 ± 155 kcal) was higher than in the continuous PA trial (2373 ± 198 kcal, $p = 0.01$). Table 3 shows RER (non-adjusted) during each segment in the chamber. 23-h RER adjusted for RER on the preceding day in the intermittent PA trial was lower than in the continuous trial ($P = 0.021$, Figure 2). Non-sleeping RER (15-h) adjusted for RER on the preceding day in the intermittent PA trial was also lower than in the continuous PA trial ($P = 0.017$). Sleeping RER adjusted for sleeping RER on the preceding day was not significantly different between trials. There was no interaction between trial and time for sleeping RER or post-breakfast RER. Although there was an effect of time between days 1 and 2 ($P < 0.001$) and a trial effect ($P < 0.01$) for sleeping RER, there was only an effect of time between days 2 and 3 ($P < 0.001$) for post-breakfast RER. 23-h fat oxidation adjusted for TEE and sleeping fat oxidation rate on the preceding day was significantly higher in the intermittent PA trial (109.8 ± 6.8 g) than in the continuous PA trial (101.1 ± 6.8 g; $P = 0.001$). There was no significant difference between trials in 23-h CHO oxidation adjusted for TEE and sleeping CHO oxidation

rate on the preceding day.

PA in the chamber.

PA in the chamber was evaluated using an accelerometer. PA during the period without respiratory measurements (0700–0800 h) on days 2 and 3 was not significantly different between trials. There was no significant difference in cycling ergometer PA with either 5.5 METs or 20 W cycling between trials, whereas non-cycling PA in the intermittent PA trial (1.33 ± 0.11 METs) was significantly higher than in the continuous PA trial (1.25 ± 0.11 METs, $p < 0.01$). The difference of means of accumulated time in trials was larger with a greater number of consecutive minutes with METs ≤ 1.5 (Table 2).

PA in the chamber and substrate oxidation.

There was no significant relationship between total minutes with METs ≤ 1.5 and 23-h RER adjusted for sleeping RER on the preceding day (Figure 3A), whereas adjusted 23-h RER was correlated with each level of accumulated consecutive minutes with METs ≤ 1.5 (3 minutes or more; $r = 0.477$, 5 minutes or more; $r = 0.510$, 10 minutes or more; $r = 0.605$, Figure 3B). Moreover, because total minutes with METs ≤ 1.5 in the intermittent PA trial was significantly lower than in the continuous PA trial, we examined the relationships between fractions of accumulated consecutive minutes with METs ≤ 1.5 relative to total minutes with METs ≤ 1.5 and adjusted 23-h RER to evaluate the influence of prolonged sedentary behavior independently

of the PA trials. The relationships were comparable for each level (3 minutes or more; r = 0.488, 5 minutes or more; r = 0.516, 10 minutes or more; r = 0.625). The mean PA intensity for non-cycling time was not significantly correlated with 23-RER adjusted for sleeping RER on the preceding day. Additionally, neither PALsleep (TEE/SMR×23/24) nor PALBMR (TEE/estimated BMR×23/24) evaluated in the respiratory chamber was significantly correlated with 23-h RER.

Blood samples.

Plasma parameters were not significantly different between trials on day 2. For all variables, no significant interaction of trial×time was observed. The only significant effect of time was between day 2 and day 3 on plasma parameters other than insulin and norepinephrine. Plasma glucose and TG decreased ($P = 0.001$, $P = 0.01$, respectively), whereas NEFA, HDL-C, and LDL-C increased ($P = 0.01$, $P < 0.05$, $P < 0.001$, respectively; Table 4).

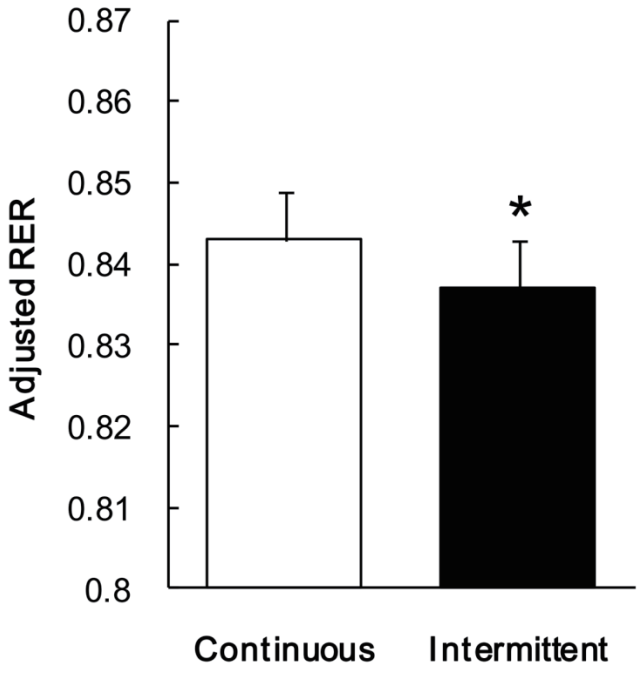

Figure 2. RER adjusted for sleeping RER for the continuous PA trial and the intermittent PA trials. Values are mean ± SD. ****P* **= 0.021 between trials.

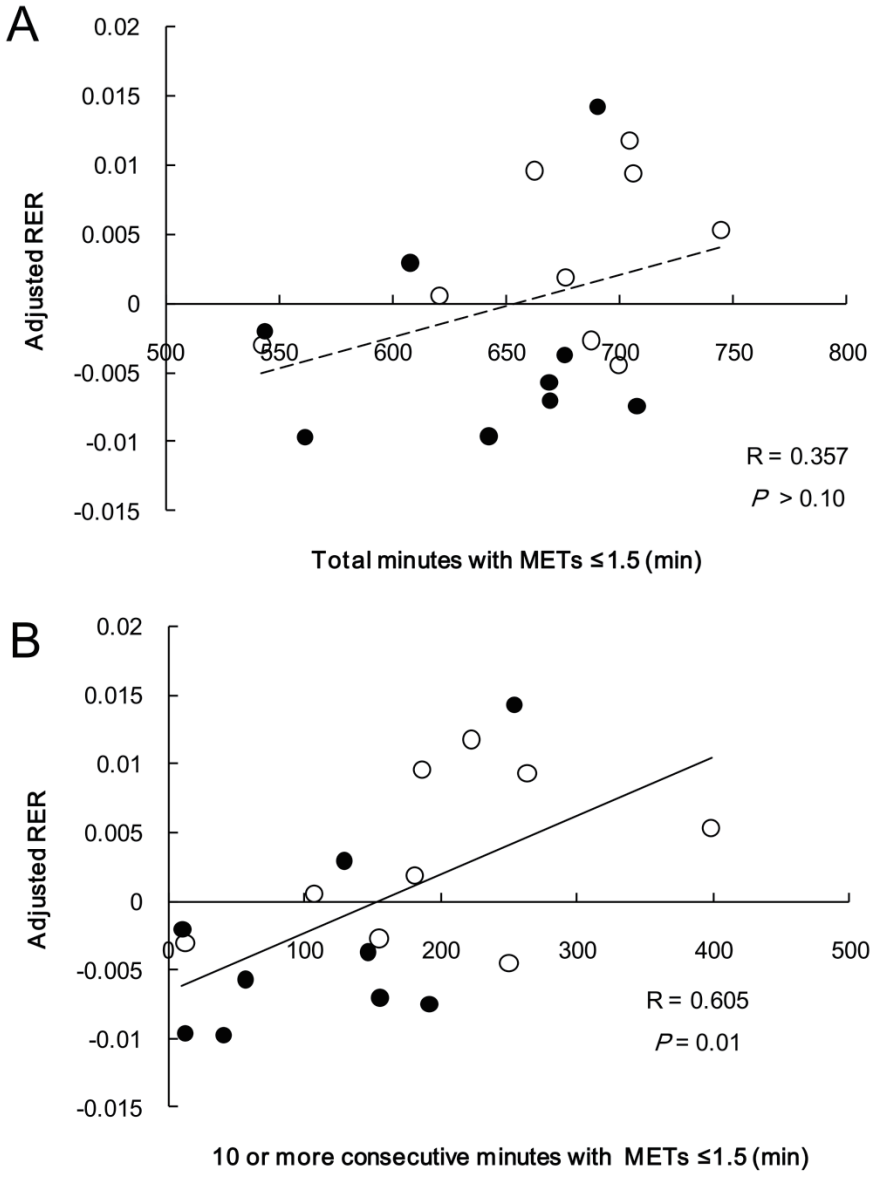

Figure 3. Relation between PA with METs ≤ 1.5 and 23-h RER adjusted for sleeping RER.

Open **circle, continuous;** ***filled*** **circle, intermittent.**

Table 4. Fasting glucose, lipid, insulin, and norepinephrine concentrations over the course of the experiment

	Continuous		Intermittent		trial	time	trial×time
	day 2	day 3	day 2	day 3			
Glucose (mg· dL^{-1})	75.1 ± 5.3	71.7 ± 3.8	76.4 ± 5.4	72.2 ± 5.0	NS	*P = 0.001*	NS
TG (mg· dL^{-1})	84.6 ± 36.7	60.0 ± 20.9	77.3 ± 31.2	60.2 ± 20.1	NS	*P = 0.01*	NS
LDL-C (mg· dL^{-1})	95.3 ± 16.1	106.6 ± 15.4	86.8 ± 12.8	97.1 ± 11.0	NS	*P < 0.001*	NS
HDL-C (mg· dL^{-1})	53.7 ± 11.4	57.7 ± 11.0	52.0 ± 15.2	54.8 ± 14.3	NS	*P < 0.05*	NS
NEFA (mEp· L^{-1})	0.314 ± 0.143	0.450 ± 0.124	0.320 ± 0.115	0.487 ± 0.09	NS	*P = 0.01*	NS
Insulin (μU· mL^{-1})	5.18 ± 1.84	5.38 ± 2.48	4.76 ± 1.40	4.86 ± 1.52	NS	NS	NS
Norepinephrine (ng· mL^{-1})	0.289 ± 0.107	0.249 ± 0.091	0.279 ± 0.105	0.234 ± 0.09	NS	NS	NS

NS, nonsignificant.

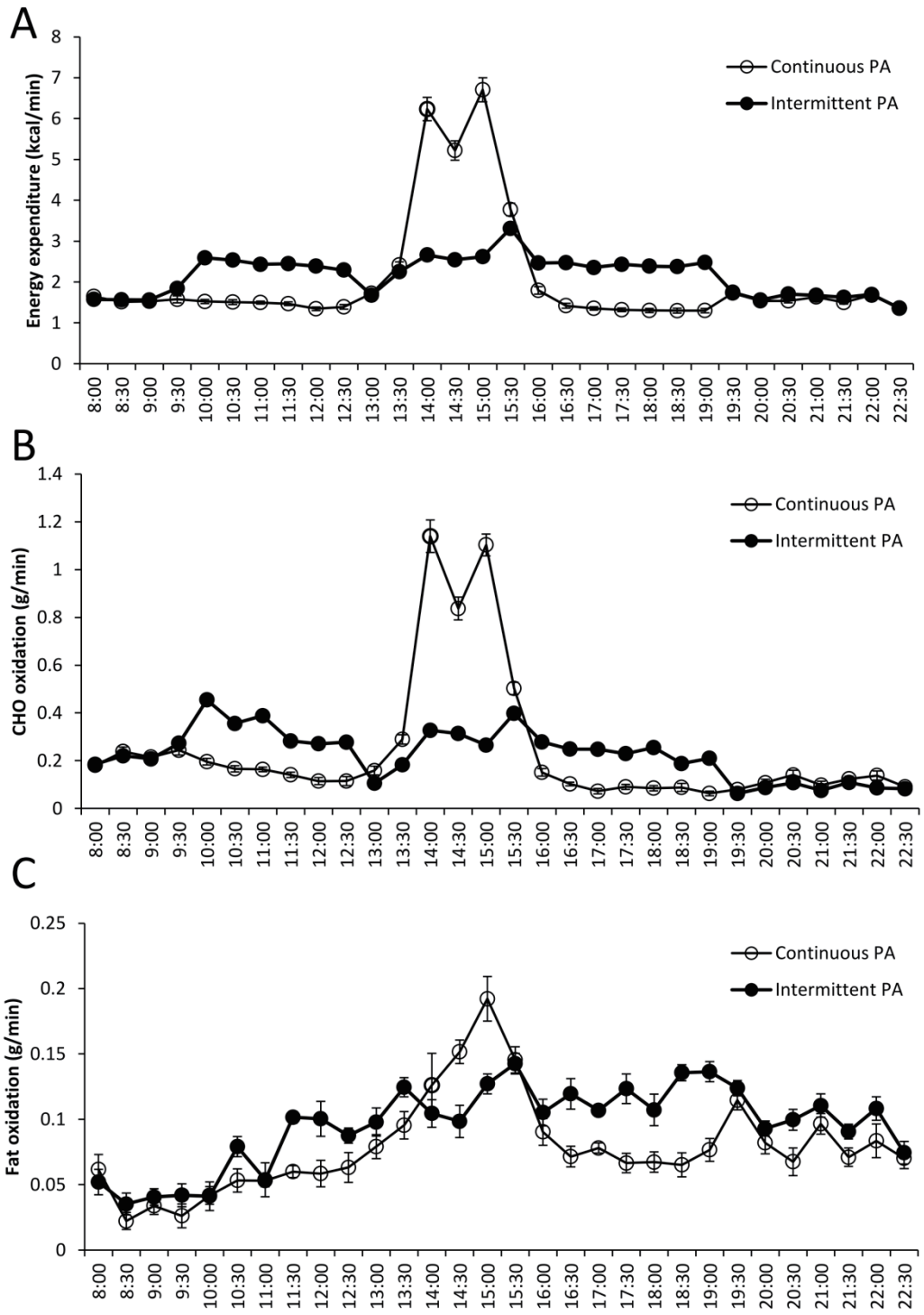

Figure 4. Mean (±SEM) energy expenditure (A) and substrate oxidation (B) (C) during awake time. *Open* circle; continuous, *filled* circle; intermittent.

DISCUSSIONS

In the present study, we examined whether continuous and intermittent moderate intensity PA differentially influence fat utilization over a whole-day. Given the results of a recent study showing that the number of “breaks in sedentary” was associated with obesity-related parameters, we hypothesized that intermittent moderate intensity PA throughout the day (e.g. walking, moving around, intense household activity, etc.) would lead to greater fat utilization than a single bout of moderate intensity PA. 23-h RER in the intermittent PA trial was significantly lower than in the continuous PA trial, although the difference in fat oxidation was only about 10g/day. In the intermittent PA trial, there were 15 more instances of standing up, moving around, and ergometer preparation than in the continuous PA trial. 23-h TEE and energy balance were significantly different between trials, but both were not significantly associated with 23-h RER in this study, although energy balance is considered as a main determinant of fat oxidation. This result may reflect that inter-individual relationships between energy balance and RER were masked by some other confounding factors. Incidentally, although the difference in TEE between trials was 83 kcal, most of this difference (70 kcal) can be explained by non-cycling PA measured by accelerometry. Additionally, PAL and Non-cycling PA (spontaneous PA) were not significantly associated with 23-h RER, although previous studies have indicated that a higher PAL leads to more rapid adaptation to an HF meal. This discrepancy

may be due to a higher PAL in both trials in the present experiment. Hansen et al. (23) also showed that the change in RER at a PAL of 1.8 was similar to that at a PAL of 1.6. Based on these results, even if PAL were the same, intermittent PA might induce greater utilization of ingested fat over the course of a day when switching from an HC meal to an HF meal than continuous PA.

It is plausible that consecutive time with METs ≤ 1.5 in the intermittent PA trial was less than in the continuous PA trial. Figure 3 shows that there was a strong correlation between 10 or more consecutive minutes with METs ≤ 1.5 and adjusted 23-h RER. Recent studies presented by Hamilton and colleagues (2, 82) suggest that prolonged sedentary behavior leads to decreased fat oxidation as a result of decreased heparin-releasable lipoprotein lipase (LPL) activity, which directs consumed fat toward muscle. Furthermore, the present study showed that the fraction of accumulated consecutive minutes with METs ≤ 1.5 relative to total minutes with METs ≤ 1.5 was strongly associated with 23-h RER adjusted for sleeping RER on the preceding day. Thus, our results may support the idea that “breaks in sedentary” prevent decreased fat oxidation.

Several studies performed under energy balanced conditions over one day showed that moderate-intensity exercise does not lead to relatively increased fat oxidation over the day (45). This phenomenon can be explained by the popular dogma that RER becomes equal to the food

quotient (FQ) in the long term. Thus, when subjects eat meals with the same macronutrient balance on pre-chamber days and chamber days, fat and CHO utilization during exercise should compensate and become equal to the FQ. However, when FQ and energy balance change from day to day, a positive fat balance is probably always easy to retain. Then, because this possibly leads to weight gain, we need to investigate those conditions that are favorable for a positive fat balance. Thus, this was the motivation for the present study. Although the difference in fat oxidation was only about 10g/day, if a positive fat balance (i.e., a negative CHO balance) accumulated rather than a threshold for stimulating appetite, this may lead to subsequent overeating. Actually, fat oxidation did not show compensation after exercise, because the measurement was performed during the period of switching from HC to HF. In addition, because LPL protein remains elevated until about 20 hours after exercise in humans (61), we continued our study until late morning on day 3. However, there were no significant interactions between pre- and post-exercise for sleeping RER or postprandial RER in the morning ($P = 0.19$, $P = 0.64$, respectively). Moreover, none of the biochemical variables on day 3 mornings showed any interactions between trials, although the sample size and only one point (fasted condition) for blood drawing were limited. Thus, excess fat oxidation after prolonged exercise during the continuous PA trial might have been completed in the morning. These data probably indicate that a positive fat balance may not be compensated and may accumulate day after day.

During switching from an HC meal to an HF meal, macronutrient utilization switches from predominantly CHO to fat until it becomes equal to the proportion of fat consumed. However, individuals cannot adapt as rapidly to changes in fat intake compared to changes in other macronutrients. More than a full day is needed to increase pyruvate dehydrogenase kinase (PDK) activity, a key factor for fat adaptation, after consumption of an HF diet (3, 52). However, prolonged low-to moderate-intensity exercise can modulate PDK activity (73). In fact, Hansen et al. (23) showed that prolonged low-intensity exercise leads to faster adaptation to an HF meal than inactivity. A previous study reported that PDK activity was modulated 4 hours after initiation of exercise. Moreover, LPL activity also began to increase 4 hours after exercise (61). These data suggest that the beneficial effects of exercise on fat metabolism in response to an HF diet require at least 4 hours. In the present study, however, increased fat utilization could be seen approximately 1–2 hours after starting exercise in the intermittent PA trial (Figure 4), although protein oxidation could not be measured. An acute negative energy balance during an intermittent PA trial compared to during a continuous PA trial might lead to accelerating fat adaptation. Thus, a synergistic effect of rapidly increased fat consumption and excess of EE by only 5 min of exercise may have contributed to the acceleration of fat adaptation in the intermittent PA trial. Therefore, intermittent PA may lead to efficient utilization of ingested fat by preventing decreased fat oxidation and accelerating increased fat oxidation for adapting to fat

consumption.

Multiple bouts of exercise have been reported to have beneficial effects for the prevention and management of obesity (21, 48), although the results of these studies were not consistent with those of another study (31). Interestingly, Goto et al. (21) showed that splitting up exercise into short sessions may be beneficial for fat utilization, although this study did not evaluate all EE and PA over an entire day. That study compared RER during the 180 minutes after either a single 30 minute bout of exercise or three 10 minute bouts of exercise with a 10 minute rest between each. The results showed that RER after intermittent exercise was lower than after continuous exercise. In the present study, RER post-dinner in the intermittent PA trial on day 2 was significantly lower than in the continuous PA trial ($P < 0.01$). However, EE post-dinner in the intermittent PA trial tended to be higher than in the continuous PA trial. Accelerometry data also showed that average PA in the intermittent PA trial (1.26 METs) was higher than in the continuous PA trial (1.21 METs) but these differences were not significant (data not shown). We speculate that higher postprandial EE in the intermittent PA trial contributed to the lower postprandial RER relative to the continuous PA trial.

There are several limitations to our study. The first limitation is that energy balance was significantly different between trials. However, EB was not significantly correlated with RER in this study. Therefore, we thought that EB did not need to be taken into consideration for

statistical analysis. However, this may reflect that a negative EB is associated with a higher RER in each participant, but these intra-individual relationships were masked in the larger inter-individual distribution with almost no relationship. Therefore, we did a correlation analysis between intra-individual differences in RER and those in EB. There was a moderate correlation between differences in (delta) RER and differences in (delta) EB ($r = 0.69$). Thus, EB appears to influence RER in each subject. However, so far as we know, these intra-individual relationships between EB and RER cannot be statistically considered for comparing 2 PA patterns without significant inter-individual relationships between EB and RER. In addition, because any consecutive minutes with sedentary behavior were not associated with energy balance (data not shown), we suggest that prolonged sedentary behavior influenced RER independently of energy balance. The second limitation is that protein consumption was used to estimate substrate oxidation. It is more accurate to estimate protein oxidation using urine samples. In the present study, however, all subjects consumed meals that contained 15% protein. Moreover, since this study used a crossover design, participants consumed the same diet in both trials. Previous studies using a similar protocol showed that protein oxidation was not different between trials, even if PAL (23) and meal frequency (62) were different. In addition, the duration of the experiment was shorter than previous studies. Previous studies have monitored fat adaptation over a 4 day period (8, 23, 63). However, participants in the present study consumed HC food

that contained 70% CHO (FQ = 0.928; higher than in previous studies) for 2 days before entering the chamber in order to detect subsequent differences in adaptation speed to an HF diet. The present data showed that average 23-h RER rapidly decreased to about 0.84, approaching the FQ of 0.827, in both trials, although daily RER during HC consumption was not confirmed. Because a previous study also reported that RER decreased substantially within one day of consuming an HF diet (23), the experimental period of the present study appears to be appropriate. In addition, we did not sequentially observe any biochemical parameters. Although mean heart rate and variables of heart rate variability, an indicator of autonomic nervous activity, were measured during experiments in the chamber, these were not significantly different between trials (data not shown), because the intensity of exercise in these experiments was low.

Traditionally, prolonged exercise for 10 minutes or more in each session has been recommended to increase EE. A study (67) and a guideline (24) for adults suggested that a certain number of consecutive minutes (≥10 minutes) of MVPA contributed to weight control more than accumulated sporadic MVPA, although this evidence was limited to cross-sectional studies. However, the prevalence of obesity has continued to increase. In our study, the intermittent PA trial, in which exercise was performed for only 5 minutes per bout, was associated with greater fat utilization than the continuous PA trial. Thus, our data strongly suggest that at least 5 or more consecutive minutes of MVPA may contribute to preventing

obesity as well as 10 or more consecutive minutes of MVPA, and may be a more achievable goal for many people, although further studies are needed to clarify the effects of PA lasting less than 5 minutes (sporadic PA). Partitioning exercise into short bouts of exercise throughout the day may be more practical for sedentary individuals who have low physical fitness.

In summary, this study provides important information about the potential impact of PA continuity on substrate oxidation over a whole-day. The present data indicate that there was greater fat oxidation in the intermittent PA trial than in the continuous PA trial after exposure to an HF meal. In addition, our data also suggest that multiple bouts of exercise for only 5 minutes promote fat utilization better than prolonged exercise. This may be explained by the fact that a greater number of consecutive minutes of sedentary behavior (METs $\leq$ 1.5) was associated with higher RER (lower fat oxidation). Thus, the present study specifically suggests that the intervals between dynamic body movements should be as short as possible for more efficient utilization of ingested fat. Whereas, because these results were obtained from only a few subjects during a short-term laboratory experiment, additional longitudinal studies and intervention studies are needed to confirm whether intermittent PA rather than continuous PA is effective for preventing obesity.

Study 2. Effects of aerobic capacity on fat utilization

INTRODUCTION

Several previous studies have indicated that a daily reduction in fat oxidation predicts weight and fat gain over a year or more (12, 13, 51, 60, 83). Utilization of dietary fat is less sensitive and adaptive compared to the other macronutrients; there is a larger capacity for fat storage, which can be expanded or contracted as required, making it a more effective depot than is the case for carbohydrate and/or protein storage. For example, “holiday weight gain” or “weekend weight gain”, binge eating at a party, or a large reduction in the level of physical activity (PA) during holidays can be the cause of a positive energy balance (EB) because these situations can increase the likelihood for ingestion of excess fat. While a “healthy” lifestyle on a daily basis is recommended in general, many people may hope to avoid or limit exercise and/or a strict diet on their days off. Therefore, optimizing the utilization of fat to help prevent weight gain under free-living conditions is most important in cases of overeating, especially if that consumption involves ingestion of high-fat foods.

In recent studies, several physiological factors have been proposed as predictors of either a lower body weight or an increase in body fat gain. For example, a higher resting metabolic rate, higher insulin secretion during oral glucose testing, and higher sympathetic activity, etc., have been reported to be associated with a subsequently lower weight or fat gain in

Pima Indians (53). However, evidence in support of such effects remains insufficient, mainly because the physiological parameters in question can be strongly influenced by genetic factors, making the parameters difficult to evaluate and improve. Aerobic capacity (AC) is also strongly influenced by genetic factors, but it is one of the most modifiable parameters in terms of the body's physiological functions, and has been used to maximize the capacity for fat oxidation. Numerous longitudinal studies have shown that a higher AC can predict lower weight gain (5, 6, 22, 33, 43, 71). Some previous studies have reported that a higher AC can predict subsequently lower weight or fat gain, even after adjustment for PA (5, 33). However, to the author's knowledge, and following an extensive search of the literature, it appears that no study sufficiently answers this question using a physiological approach: why can AC predict subsequently lower weight or fat gain.

Many studies focus on fat utilization during exercise and show that a higher AC leads to more fat utilization under identical workloads. This outcome does not change, even when relative intensity or maximum oxygen uptake are the same (66, 81); the only exception may be in men, for whom the evidence is conflicting (16, 17). There are only a few studies that focus on fat utilization in conjunction with some physical activity over a whole day (36, 44, 56, 59, 64). More study results showed that AC does not result in fat utilization and only a minority of studies reported a positive association between the two. Smith et al. described that

higher ACs result in much more fat utilization under conditions in which fat tends to be in excess, PA level (PAL) is low (PAL = 1.4) and the diet has been switched from being high-carbohydrate (HC) to high-fat (HF). A PAL of 1.4 indicates that free-living individuals would be well-advised to engage in very low-intensity activity throughout the day. In the Smith et al. study, participants performed prolonged exercise for a total of 30 minutes, but this was done in a sporadic manner 2 or 3 times a day for at least 10 minutes per session in a human calorimeter to maintain the PAL of 1.4. There were some potential limitations associated with the Smith study. First, it is uncertain whether or not prolonged exercise served as an influential stimulus to the relationship between AC and fat utilization. Second, it is unclear whether or not the continuity or sporadic nature of the exercise influenced the relationship between AC and fat utilization. Third, there is doubt as to it whether or not the relationship between AC and fat utilization took into account the sedentary periods pre- and post-exercise or just considered the exercise period. As a result, it may be conceivable that higher AC leads to higher metabolic flexibility during physical inactivity; however, there is no definitive evidence.

The primary purpose of the present study was to determine whether there is a relationship between AC and fat utilization; this is based on the rationale that there are few studies and the results are largely inconsistent. An investigation was also conducted to determine whether the continuous or sporadic nature of exercise influences the relationship

between AC and fat utilization. The final aim of this study related to determining whether there was a relationship between AC and fat utilization during sedentary periods pre- and post-exercise.

RESULTS

The relationship between AC and energy substrate utilization

Table 5 and Figure 5 show the relationship between AC and the respiratory exchange ratio (RER) during the continuous and intermittent trials. There was a significant relationship between all parameters of AC and non-sleeping time (15-h) RER, and also between AC and whole-day RER (23-h) in both trials. Because there was a possibility of an association between AC and RER for sleeping time on day 1, sleeping time RER was analyzed as a covariate. All parameters of AC were strongly correlated with non-sleeping time (15-h) RER, and whole-day RER (23-h) in the continuous PA trial adjusted for sleeping RER on the preceding day. On the other hand, in the intermittent PA trial, no parameters of AC were significantly correlated with non-sleeping time (15-h) RER, although many parameters of AC were significantly correlated with whole-day RER (23-h) in the intermittent PA trial adjusted for sleeping RER on the preceding day. Because the slopes for both trials were comparable, these analyses were performed together with an adjustment made for trial. All parameters of AC were significantly correlated with non-sleeping time (15h) RER, and whole-day RER (23-h) adjusted for sleeping RER on the preceding day and trial. There was no significant relationship between any parameter of AC and sleeping RER on day 2.

Table 5. Correlation coefficients between AC and RER in continuous and intermittent physical activity trial segments

	Continuous PA trial				Intermittent PA trial				non-sleep adjusted for trials	sleep day 2 adjusted for trials	whole day adjusted for trials
	sleep day 1 2305-0700 h	non-sleep 0800-2300 h	sleep day 2 2305-0700 h	whole day 0800-0700 h	sleep day 1 2305-0700 h	non-sleep 0800-2300 h	sleep day 2 2305-0700 h	whole day 0800-0700 h			
VO2peak (ml· min^{-1})	-0.464	-0.704*	-0.488	-0.723*	-0.604	-0.700*	-0.351	-0.697*	-0.701**	-0.418	-0.710**
VO2peak adjusted for sleeping RER (ml· min^{-1})	-	-0.733*	-0.234	-0.880**	-	-0.444	0.136	-0.438	-0.582*	-0.067	-0.635**
VO2peak (ml· kg weight^{-1}· min^{-1})	-0.368	-0.679*	-0.157	-0.644	-0.496	-0.704*	-0.193	-0.688*	-0.691**	-0.158	-0.666**
VO2peak adjusted for sleeping RER (ml· kg weight^{-1}· min^{-1})	-	-0.845**	0.207	-0.868**	-	-0.641	0.259	-0.613	-0.733**	0.233	-0.721**
VO2peak (ml· kg FFM^{-1}· min^{-1})	-0.472	-0.744*	-0.260	-0.720*	-0.570	-0.737*	-0.275	-0.723*	-0.740**	-0.249	-0.722**
VO2peak adjusted for sleeping RER (ml· kg FFM^{-1}· min^{-1})	-	-0.822*	0.176	-0.855**	-	-0.596	0.223	-0.568	-0.699**	0.201	-0.692**
Percentage of VO2peak at 5.5 METs (%)	0.413	0.711*	0.205	0.678*	0.553	0.763*	0.204	0.744*	0.737**	0.192	0.711**
Percentage of VO2peak at 5.5 METs adjusted for sleeping RER (%)	-	0.843*	-0.189	0.863**	-	0.688	-0.319	0.653	0.757**	-0.243	0.740**

***P* < 0.05; ****P* < 0.01. Abbreviations: PA, physical activity; FFM, fat-free mass; RER, respiratory exchange ratio; VO2peak, peak oxygen consumption.**

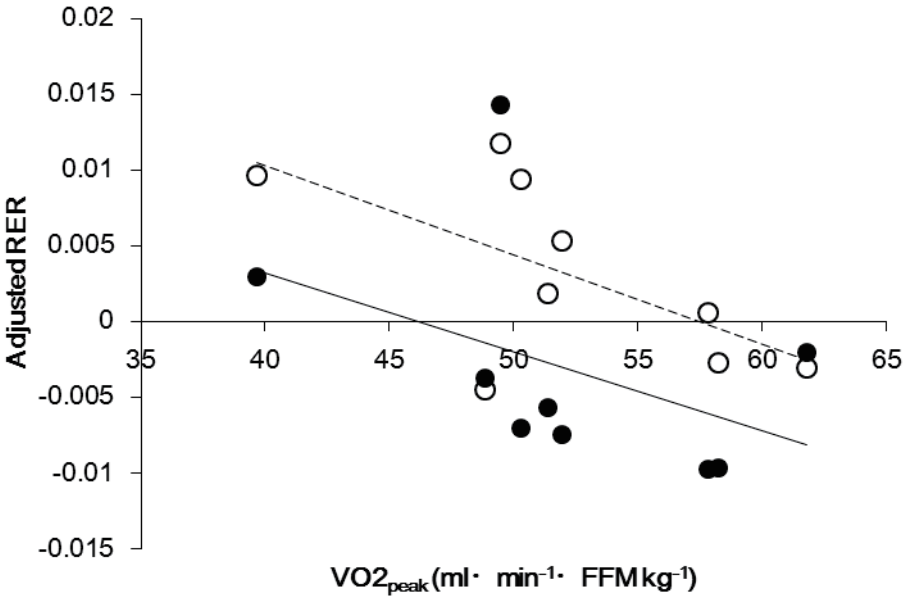

Figure 5. Relationship between AC and 23-h RER adjusted for sleeping RER.

open circle, continuous physical activity (PA); *filled* circle, intermittent PA; *solid* line, regression line in continuous physical activity trial; *dashed* line, regression line in intermittent physical activity trial. Abbreviations: AC, aerobic capacity; FFM, fat-free mass; RER, respiratory exchange ratio; VO2peak, peak oxygen consumption.

The relationship between AC and energy substrate utilization during prolonged pre- and post-exercise periods during the continuous PA trial

Table 6 and Figure 6 show the relationship between AC and RER during sedentary periods in the continuous PA trial. All parameters of AC were strongly correlated with the RER during the exercise period in the continuous PA trials. However, there was no significant relationship between parameters of AC and the pre-exercise period in the continuous PA trials, whereas all parameters of AC were associated with the post-exercise period RER in the continuous PA trials.

Table 6. Correlations between AC and RER before, during, and post-exercise in the continuous PA trial

	Continuous PA trial		
	0800-1350 h	1350-1550 h	1550-2300 h
VO2peak ($ml \cdot min^{-1}$)	-0.482	-0.773*	-0.775*
VO2peak adjusted for sleeping RER ($ml \cdot min^{-1}$)	-0.146	-0.719*	-0.776*
VO2peak ($ml \cdot kg\ weight^{-1} \cdot min^{-1}$)	-0.423	-0.778*	-0.617
VO2peak adjusted for sleeping RER ($ml \cdot kg\ weight^{-1} \cdot min^{-1}$)	-0.263	-0.806*	-0.591
VO2peak ($ml \cdot kg\ FFM^{-1} \cdot min^{-1}$)	-0.489	-0.823**	-0.697
VO2peak adjusted for sleeping RER ($ml \cdot kg\ FFM^{-1} \cdot min^{-1}$)	-0.145	-0.798*	-0.613
Percentage of VO2peak at 5.5 METs (%)	0.475	0.817**	0.660
Percentage of VO2peak at 5.5 METs adjusted for sleeping RER (%)	0.304	0.830*	0.615

*** $P < 0.05$; ** $P < 0.01$. Abbreviations: PA, physical activity; FFM, fat-free mass; RER, respiratory exchange ratio; VO2peak, peak oxygen consumption.**

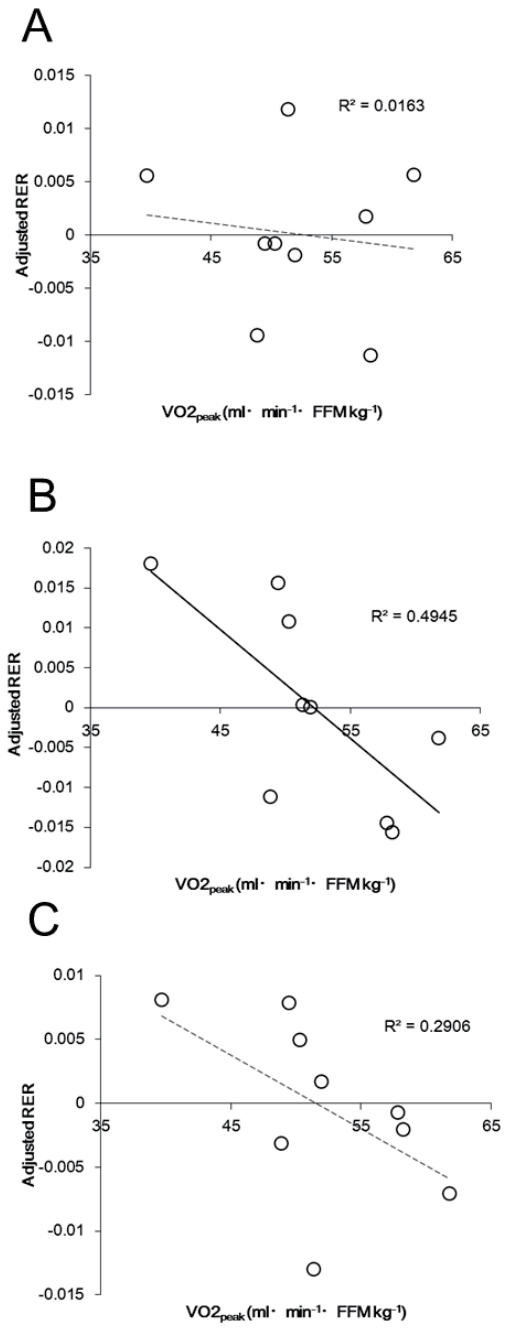

Figure 6. Correlations between AC and RER before exercise (A), during exercise (B), and post-exercise (C) during the continuous PA trial.

Abbreviations: AC, aerobic capacity; FFM, fat-free mass; RER, respiratory exchange ratio; VO2peak, peak oxygen consumption.

DISCUSSION

To prevent weight or fat gain in free-living individuals, it may be most important to optimize fat utilization in cases of overeating, especially if fat is consumed in excess. For this reason, it is recommended that a relatively "healthy" lifestyle should be adopted, even during a holiday(s). However, many people may be less interested in performing exercise during a day off, and they may also be less inclined to want to adhere to dietary restrictions. Considering the possibility of these tendencies, it is beneficial if ingested fat can be easily utilized; this rationale is the basis for the focus of this study that investigated the influence of AC on fat utilization. The results of this study show that higher AC was associated with higher fat utilization during trials of continuous and intermittent physical activity when diets were switched from HC to HF yielding results similar to those of Smith et al. (64). Thus, the results of our study support previous evidence. Furthermore, this study was an investigation to determine whether the PA pattern is influential, in relation to exercise continuity in particular. The results of this study suggest that the associations identified in the continuous PA trial were stronger than those in the intermittent PA trial; however, it is not possible to elaborate on statistical predominance in the present study, because the slopes of the regression lines in the both trials were almost the same. It is notable that the present results were not in agreement with Roy's study (56) performed in patients consuming an HF diet. This disparity in outcomes might be due to the difference in

exercise intensity in the chamber. Although our study and Smith's study were based on exercise approximating 5METs on the subjects, Roy's study imposed exercise of less than 3METs. Thus, relatively intense PA may be required to maintain the relationship between AC and fat utilization.

All parameters of AC were strongly correlated with RER during the exercise period in the continuous PA trials, even if the RER was adjusted for the sleeping RER on the preceding day. Many studies support this result showing that a higher AC results in increased fat utilization under conditions of the same workload (16, 17, 66, 81). This investigation also determined whether there was a relationship between AC and fat utilization during sedentary periods pre- and post-exercise in the continuous PA trial. All parameters of AC tended to be associated with the post-exercise period RER after adjustment for sleeping RER in the continuous PA trials, although the *P* values were marginal. The results of one study participant appeared to be an outlier, and after removing that participant's data, there was an extremely strong correlation between AC and RER adjusted for sleeping RER on the preceding day ($R = 0.97$, Figure C). Thus, a strong association between AC and 23-h RER might exist. Because prolonged lower-intensity exercise can modulate pyruvate dehydrogenase kinase (PDK) activity (73), higher AC may accelerate fat adaptation after exercise.

Results of the study showed there was no significant relationship between all parameters of AC and the pre-exercise period RER in the continuous PA trials, although unadjusted RERs tended to be associated with AC. Thus, there may be no benefit of higher AC on fat utilization during sedentary periods. More specifically, the pre-exercise RER was associated with AC, but only if no adjustment was made. If the influences of substrate utilization during sleep persist in the daytime and aid in the utilization of ingested fat, the relationship between AC and unadjusted RER in the pre exercise period should represent just one benefit of a higher AC on fat utilization. Recent studies in the field of obesity and diabetes have addressed metabolic flexibility. Metabolic flexibility is the capacity of the body to match fuel oxidation to ingested fuel (34). In reference to the aforementioned definition, Galgani et al. (19) stated that, "*... the switch from carbohydrate to lipid oxidation during an overnight fast should also be part of the assessment of metabolic flexibility.*"

Most previous studies have demonstrated no association between substrate utilization during sleep and subsequent weight gain (13, 74-76), whereas other studies have shown the association between non-sleeping time or 24-h substrate oxidation and weight gain (13, 83).Thus, utilization of ingested fat during the daytime may be an important factor in the prevention of obesity. Enhancement of PA should also be considered important for utilization of fat and prevention of subsequent weight or fat gain. However, because these results were

obtained following only a few hours of physical inactivity during pre-exercise, additional studies and intervention studies examining participants under conditions of physical inactivity over a whole day are needed to confirm whether there is the relationship between AC and fat utilization during physical inactivity.

Comprehensive discussion and summary

A large body of evidence supports the notion that habitual exercise, higher PAL, and well-balanced food intake is generally required to prevent of obesity. However, for many people who have a very busy and fast-paced lifestyle, it can be an everyday challenge to follow dietary and lifestyle recommendations, although they may even acknowledge it can help to prevent weight/fat gain. Consequently, the increasing prevalence of obesity will likely continue. This research has focused on how ingested fat predicts subsequent weight/fat gain in free-living conditions and shows how outcomes are dependent on individuals' behaviors.

Study 1 demonstrated the effect of PA on fat utilization. In fact, expending as much energy as possible is most important in terms of the utilization of fat. Longitudinal data have shown the amount of PA cannot fully explain recent weight gain in adults. These findings suggest that it is highly possible that other factors related to PA, but independent of PAL, may influence weight and fat gain in adults. Study 1 indicated that fat oxidation increased more in participants in the intermittent PA trial than in the continuous PA trial after exposure to an HF meal. In addition, our data also suggested that multiple bouts of exercise, even if only performed for a duration of 5 minutes, did more to promote fat utilization than did prolonged exercise. This may be explained by the fact that longer periods of sedentary behavior (consecutive minutes at METs $\leq$1.5) were associated with higher RER (lower fat oxidation). The

present study specifically suggests that the intervals between dynamic body movements should be as short as possible for more efficient utilization of ingested fat. This study also provides important information about the potential impact of PA continuity on substrate oxidation over a whole day. Therefore, on days when excess fat is ingested, in particular on holidays, occasionally breaking the sedentary pattern of no body movement may help with the increased utilization of ingested fat. In addition, partitioning exercise into short sessions throughout the day may be a more practical approach to PA for sedentary individuals who tend to have lower levels of physical fitness.

Study 2 was an investigation of the effect of physiological function on fat utilization. Optimizing the utilization of fat to help prevent weight/fat gain under conditions of free-living is most important in cases of overeating, especially if that consumption involves ingestion of high-fat foods; the utilization of ingested fat via body functions should not be difficult. Because some longitudinal studies reported that higher AC can lead to lower weight gain, this investigation was designed to determine whether or not higher AC helps to utilize ingested fat over a whole day. A key point of focus was the relationship between AC and fat utilization during a period of physical inactivity pre- and post-exercise. The results of Study 2 indicated that there was strong association between AC and fat utilization after exposure to a HF meal in both the continuous and intermittent PA trials; higher AC led a greater fat utilization, especially

in the continuous PA trial. This may be explained by the fact that the strong relationship between AC and RER continued after exercise. However, there was poor relationship between AC and RER in the physical inactivity period before exercise. This study therefore suggests that AC does not help to prevent an excess of fat gain when individuals remain inactive for long periods, such as during prolonged sitting.

In summary of the results described above, it is important that the intervals between dynamic body movements be as short as possible to more efficiently utilize ingested fat, especially during a holiday period when an individual may be less active than usual and the consequence may result in a gain of weight/fat.

The cause of overeating can be very complex, even though extensive scientific research has been performed to increase our understanding of its etiology For example, beyond the biological factors that influence overeating are psychological and social issues such as the gender difference which can provoke appetite and overeating. Previous studies have reported that eating behavior is a very strong predictor of adult weight gain, in spite of the controversial predictive value of such dietary composition variables as percentage of energy derived from fat, carbohydrate, and protein. In particular, “disinhibition” has been shown to be strongly associated with weight gain over 20 years in adult life. Flexible, but not rigid, control of dietary restraint might attenuate the influence of habitual disinhibition on weight gain in women (10, 25,

26). However, the evidence in men does not support the association between initial restrained and subsequent weight gain (9, 10). Additional studies are needed to confirm the inter-individual variability of weight/fat gain and the effective preventative care for obesity. Since clarifying the complicated cause of obesity is important, we should not forget that there may be an easy way, as mentioned by Bray et al. (4) who stated that, "*...perhaps best way to maintain weight over a long period is not counting kilocalories, but weighting oneself regularly at the same time of day on an accurate scale, and then decreasing food intake or increasing activity if weight has been gained.*"

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Publication List

This dissertation is based on the following original research article:

Ando T, Usui C, Ohkawara K, Miyake R, Miyashita M, Park J, Ezaki O, Higuchi M, Tanaka S. Effects of intermittent physical activity on fat utilization over a whole day. *Med Sci Sports Exerc*. 2013;45(7):1410-8.

Additional published article:

Hibi M, **Ando T**, Tanaka S, Tokuyama K. Human calorimetry: energy expenditure and substrate utilization measurements using a respiratory chamber. *J Phys Fitness Sport Med*. 2013; 2(1):93-9.