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ORIGINAL ARTICLE

Physical activity level in healthy free-living Japanese estimated by doubly labelled water method and International Physical Activity Questionnaire

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Objective: To measure total energy expenditure (TEE) for normal healthy Japanese by the doubly labelled water (DLW), and to compare the physical activity level (PAL) among categories classified by the categories used in daily reference intake (DRI), Japan and the International Physical Activity Questionnaire (IPAQ).
Subjects and methods: A total of 150 healthy Japanese men and women aged 20- to 59-year-old living in four districts of Japan. TEE was measured by the DLW method, and the PAL was calculated from TEE divided by basal metabolic rate. Simultaneously with TEE measurement, the PAL was assessed employing the categories used in DRI, Japan and IPAQ.
Results: The average TEE and PAL were 10.78 ± 1.67 MJ/day and 1.72 ± 0.22 for males and 8.37 ± 1.30 MJ/day and 1.72 ± 0.27 for females, respectively. The subjects in the highly active categories assessed by both DRI and IPAQ showed significantly higher PAL compared with less active categories. However, PALs among light and moderate categories by DRI, and insufficient and sufficiently active by IPAQ were not significantly different.
Conclusions: In developed countries, highly active subjects could be assessed by a simple questionnaire. However, the questionnaire should be improved to clarify the sedentary to moderately active subjects by assessing carefully very light to moderate physical activity.
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Introduction

Assessment of total energy expenditure (TEE) is essential for establishing dietary reference intakes (DRI) and recommendations for physical activity. The doubly labelled water (DLW) method is recognized as the gold standard for measuring TEE in free-living conditions (Montoye *et al.*, 1996). Many studies using the DLW method have been performed, mainly in developed countries (Schulz *et al.*, 1994; Black *et al.*, 1996; Prentice *et al.*, 1996; Westerterp, 2003; Brooks *et al.*, 2004). However, the physical activity level (PAL) calculated as TEE divided by the basal metabolic rate (BMR) is expected to be different among populations with different lifestyles. The typical lifestyle of healthy

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Japanese may have different amounts and types of physical activities compared with inhabitants of western countries. For example, many Japanese adults take trains or buses with walking to and from the stations or bus stops to work on weekdays, spending a relatively longer time commuting with a mean time of about 80 min/day on average (NHK Broadcasting Culture Research Institute, 2001). There are few people with body mass index (BMI) of 30 or more (0.8%) according to the National Nutrition Survey in Japan, 2003 (Ministry of Health, Labour and Welfare, Japan, 2006), categorized into obesity by the WHO classification (World Health Organization, 1997). The assessment of the PAL among normal healthy Japanese will serve as valuable data to consider the appropriate amount of physical activity. Then the primary purpose of the present study is to measure TEE for normal healthy Japanese living in four districts of Japan, chosen from sex and age categories.

Several indirect methods, for example, activity records, heart rate monitoring and accelerometer methods, have been used for estimating daily energy expenditure (Lamonte and Ainsworth, 2001; Vanhees *et al*., 2005). The factorial methods and indirect measures, even if done well, provide estimates that are not sound and often inaccurate. However, a simple questionnaire to assess the PAL is required when we use DRI or provide recommendations for physical activity in the practical field of public health or epidemiological study with a larger sample. The second objective of this study is to compare the PAL among the categories classified according to the DRI in Japan (Ministry of Health, Labour and Welfare, Japan, 1999) and the International Physical Activity Questionnaire (IPAQ) (Murase *et al*., 2002; Graig *et al*., 2003) to develop a simple way to categorize the PAL.

Subjects and methods

Subjects

Study participants were Japanese men and women who were recruited from Kagoshima, Niigata, Fukuoka and Tokushima Prefectures in Japan. Subjects were recruited through health care centres in each prefecture or at four workplaces. In each location, five subjects from each sex and age category (20–29, 30–39, 40–49 and 50–59 years) were selected according to the following criteria: (1) in good health, (2) not pregnant or breast-feeding, (3) BMI less than 30 kg/m^2, (4) lived in their home prefecture 2 weeks before and during the study, (5) not on a weight-loss or treatment diet, (6) did not consume more than 40 g of alcohol per day and (7) did not engage in a physically demanding occupation. However, we could not select the subjects randomly from different levels of physical activity. One hundred and fifty-seven subjects volunteered for the present study. Data were collected from May to August 2003. Over the whole assessment period, subjects were carefully instructed to maintain their normal daily activities and eating patterns and to make no conscious effort to lose or gain weight.

Study protocol

This study was approved by the Ethical Committee of the National Institute of Health and Nutrition in Japan. All subjects gave their informed consent before the commencement of the investigations. TEE was estimated over the 14-day study period in free-living conditions using the DLW method. Body weight and height were measured in the fasting state before the dose of DLW and the last day of the study. To assess the food quotient (FQ) and their PAL, a self-administered diet history questionnaire (DHQ) and a questionnaire on physical activity were completed for all subjects before and after the study period. In this study, the questionnaire assessed before the study was used in the analysis. Diet history was asked using the DHQ (Sasaki *et al*., 1998a, b). The DHQ is a validated 16-page questionnaire that recalls dietary habits over a 1-month period. Physical activity status was assessed using the last 7-day short version of the IPAQ Japanese version (Murase *et al*., 2002; Graig *et al*., 2003). Subjects were divided into three categories according to the IPAQ Scoring Protocol (Graig *et al*., 2003). In addition, the total metabolic equivalents (total METs) were calculated as the sum of walking time multiplied by 3.3, the time of moderate activity multiplied by 4.0 and the time of vigorous activity multiplied by 8.0. The physical activity status was also assessed by the category used in the DRI, Japan sixth edition (Ministry of Health, Labour and Welfare, Japan, 1999).

DLW energy measurement

After providing a baseline urine sample, a single dose of approximately 0.06 g/kg body weight of ^2H_2O (99.8 atom%, Cambridge Isotope Laboratories, Andover, MA, USA) and 0.14 g/kg body weight of $H_2^{18}O$ (10.0 atom%, Cambridge Isotope Laboratories) were given orally to each subject using a straw. Next, the container was rinsed twice with 50 ml of tap water provided from the same place where the subject lived. After dose administration, the subject refrained from eating and drinking over a 4-h equilibration period (4 h sampling) for measurement of total body water (TBW). Then, the second voided urine on the mornings of day 1 (the next day of DLW dose) and day 14 (at the same time as the void of day 1) was collected for the isotopic (^2H and ^{18}O) elimination rate. All urine samples except for baseline were collected by the participant, and the time of sampling was recorded. All samples were first stored by freezing at −40°C in airtight parafilm-wrapped containers, and then transported to the analytical facility for isotopic abundance analyses.

Gas samples for the Isotopes Ratio Mass Spectrometer (IRMS) were prepared by equilibration of urine sample with a gas. The gas for equilibration of ^{18}O was CO_2 and that for ^2H was H_2. Pt catalyst was used for equilibration of ^2H. The isotopic analyses were conducted using machines of IRMS of DELTA Plus (Thermo Electron Corporation, Bremen, Germany) calibrated using Vienna Standard Mean Ocean

Water, 302B and Greenland Ice Sheet Precipitation standard provided from International Atomic Energy Agency. Each sample and the corresponding reference were analyzed in duplicate. The average standard deviations through the analyses were 0.5‰ for ^2H and 0.03‰ for ^{18}O. The difference in the two repeat measurements of the 10 same sets of urine samples was 1.6±3.9%. TEE was expressed as the mean TEE over the 13-day period of assessment.

Analytical calculations of isotopic abundance and TEE
The dilution space of each subject was obtained from urine (^2H and ^{18}O) enrichments using the following equation (Racette *et al.*, 1994).

$$N = [WA(\delta a - \delta t)]/[18.02a\,(\delta u - \delta b)]$$

where N (mol) is the dilution space, W (g) is the amount of tap water used to dilute the dose for analysis, A (g) is the amount of dose given to the subject, a (g) is the amount of dose diluted for analysis and δ (‰) is the isotopic abundance of the dose (a), tap water (t), urine sample at 4 h after dose (u) and baseline urine (b).

TBW (mol) was calculated as the mean of Nd (mol) divided by 1.041 for dilution space estimated by ^2H and No (mol) divided by 1.007 for dilution space estimated by ^{18}O.

rCO_2 were determined from the next equation.

$$rCO_2 = 0.4554 \times TBW \times (1.007\,k_O - 1.041\,k_H)$$

where rCO_2 (mol/day) is the CO_2 production rate, TBW (mol) is the total body water, and k_O (per day) and k_H (per day) are the elimination rates of ^{18}O and ^2H, respectively (Wolfe, 1992; Racette *et al.*, 1994).

Each elimination rate (k) was calculated as follows:

$$k = [\ln(\delta_f - \delta_b) - \ln(\delta_i - \delta_b)/t]$$

where δ_i and δ_f are the isotopic abundance of the urine samples collected after dose administration on day 1 and the final day (day 14) of the assessment period, respectively; δb is the isotopic abundance of the urine sample background (baseline sample); and t represents the duration of the assessment period in days, which came to 13 in the present study.

Finally, TEE (kcal/day) calculation was performed using a modified Weir's formula (Weir, 1949) based on rCO_2 (mol/day) and FQ. FQ is calculated from DHQ, and average value of all present subjects (0.867±0.03) was used in this calculation. This assumes that under conditions of perfect nutrient balance, the FQ must equal the respiratory quotient (RQ) (Black *et al.*, 1986; Jones and Leitch, 1993; Surrao *et al.*, 1998).

$$TEE = 3.9 \times (rCO_2/FQ) + 1.1 \times (rCO_2)$$

PAL was calculated to be TEE/BMR. BMR was estimated according to the sixth Recommended Dietary Allowances for Japanese (Ministry of Health, Labour and Welfare, Japan, 1999).

Statistics
Statistical analyses were performed with SPSS for Windows (version 13.0J; SPSS Inc., Chicago, IL, USA). All results are shown as mean±s.d. The comparison of TEE and PAL in sex, age and area was tested by three-way analysis of variance (ANOVA). The PAL in the categories of physical activity assessed by questionnaire was compared by one-way ANOVA. All statistical tests were regarded as significant when the $P<0.05$.

Results

Of the 157 subjects who participated in this study, 150 were included in the analytic sample. Seven subjects were excluded because urine samples were not collected or kept properly.

Physical characteristics of all present subjects are shown in Table 1. Changes in body weight during the study period were −0.5 to 0.1 kg in each sex and age group. Males in their 30s and 40s decreased significantly body weight during the study period; however, their changes were within 3% of body weight at pre-examination. Of all the subjects, 6.8% of males and 13.2% of females were classified as lean (BMI less than 18.5 kg/m^2) and 36.5% of males and 14.5% of females were classified as obese (BMI more than 25 kg/m^2) according to the criteria for Japanese (Japan Society for the Study of Obesity, 2006). The average TBW was 36.9±4.8 kg for males and 27.2±35 kg for females. If we used 73.2% for the proportion of water in fat mass (Heyward and Wagner, 2004), the percent of fat mass was 24.7±6.0% for males and 31.4±5.7% for females.

Mean values of TEE and PAL were presented for each sex and age group in Table 2. The average TEE and PAL were 10.78±1.67 MJ/day and 1.72±0.22 for males, 8.33±1.31 MJ/day and 1.72±0.27 for females, respectively. The minimum of the average PAL values in sex and age groups was 1.58±0.29 for females in their 20s and the maximum was 1.78±0.20 for 30-year-old males. PAL for 20- to 29-year olds showed lower levels than the other age groups; however, there were no significant differences in TEE and PAL among age groups, sexes and areas.

Table 3 shows TEE and PAL among four categories assessed by DRI, Japan. The distribution of four categories across sex and age groups was uniform. Categories III (light heavy) and IV (heavy) had relatively higher PAL compared with categories I (light) and II (moderate). When we combined categories III and IV together ($n=10$, PAL$=1.87\pm0.29$) because of their small number, this category had significantly higher PAL compared with category I ($P=0.036$).

Table 4 shows TEE and PAL across the three categories assessed by IPAQ. The distribution of these three categories

Table 1 Physical characteristics of all subjects

Age group	n	Age (year)	Height (cm)	Weight (kg)				BMI (kg/m^2)	TBW (kg)
				Pre	Post	Difference	P^a		
Male									
20–29	19	25.1±2.7	171.2±6.1	65.0±11.3	64.8±11.0	−0.2±1.0	0.354	22.1±3.0	38.1±5.3
30–39	18	33.8±3.3	168.9±5.2	67.4±10.7	66.9±10.6	−0.5±0.7	0.012	23.6±3.7	36.0±4.9
40–49	18	43.8±2.5	170.4±7.5	70.8±8.9	70.3±8.8	−0.5±0.6	0.008	24.4±2.6	37.9±4.6
50–59	19	53.3±2.5	166.5±5.4	67.5±7.9	67.3±7.8	−0.2±0.8	0.415	24.3±2.4	35.5±3.9
Total	74	39.0±11.1	169.2±6.3	67.6±9.8	67.3±9.7	−0.3±0.8	0.001	23.6±3.0	36.9±4.8
Female									
20–29	17	24.9±2.7	160.6±7.2	54.1±8.9	53.9±9.0	−0.2±0.6	0.303	20.9±3.0	27.8±3.9
30–39	22	33.7±2.8	159.6±4.3	55.0±8.0	55.1±8.2	0.1±0.8	0.705	21.6±3.0	28.0±3.9
40–49	22	44.0±3.0	157.0±6.1	53.9±7.4	53.9±7.6	−0.1±0.7	0.669	21.9±2.8	27.0±3.2
50–59	15	52.7±2.0	153.9±4.5	53.9±4.9	53.9±4.7	0.1±0.5	0.712	22.7±1.5	2.55±2.2
Total	76	38.5±10.2	157.9±6.0	54.3± 7.4	54.2± 7.5	0.0±0.7	0.734	21.8±2.7	27.2±3.5

Abbreviations: BMI, body mass index; TBW, total body water by doubly labelled method.
Values are means±s.d.
[a]*P*-value for paired *t*-test for body weight at pre- and post-examination.

Table 2 TEE and PAL by sex and age group

Age group	N	TEE (MJ/day)	PAL
Male			
20–29	19	11.01±1.56	1.72±0.29
30–39	18	11.11±2.20	1.78±0.20
40–49	18	10.80±1.52	1.67±0.20
50–59	19	10.23±1.30	1.71±0.14
Total	74	10.78±1.67	1.72±0.22
Female			
20–29	17	8.29±1.51	1.58±0.29
30–39	22	8.53±1.65	1.76±0.29
40–49	22	8.40±0.98	1.75±0.22
50–59	15	8.17±0.92	1.77±0.22
Total	76	8.37±1.30	1.72±0.30

Abbreviations: PAL, physical activity level; TEE, total energy expenditure.
Sex difference: $P=0.799$.
Age group difference: $P=0.196$.
Area group difference: $P=0.336$.

was not significantly different across sex and age groups. The insufficiently active (category I) and the sufficiently active (category II) groups had significantly lower PAL than the highly active group (category III), though there were few in the highly active group (category III). However, PAL did not differ significantly between the insufficiently active and the sufficiently active categories. Farther, we divided the subjects equally among the three groups according to the total METs assessed by IPAQ and PAL measured by the DLW method, respectively. As the results, only 36% of the subjects were classified into the same level of groups by both IPAQ and DLW data, 31% of them were classified in the lower groups and another 33% were classified into the higher groups divided by IPAQ compared with groups divided by PAL measured by the DLW method.

Discussion

In the present study, average PAL was 1.72 for males and 1.71 for females, respectively. When we compared PAL among the physical activity categories assessed by DRI, Japan and IPAQ, highly active groups showed significantly higher PAL; however, PAL in the lowest and moderate groups did not differ significantly.

The overall average PAL in the present study was similar to the average PAL for the general population of western countries (Schulz *et al*., 1994; Black *et al*., 1996; Prentice *et al*., 1996; Westerterp, 2003), but relatively higher than the sedentary Japanese in the previous studies (Ebine *et al*., 2002; Peng *et al*., 2005). Ebine *et al*. (2002) reported PAL of 1.63 for 10 Japanese male students (24.2±1.8 years), and Peng *et al*. (2005) reported that of 1.62 for middle-aged sedentary women (49.4±6.0 years). We measured previously TEE for simulated sedentary lifestyle according to the data on NHK's National time use survey (NHK Broadcasting Culture Research Instutute, 2001) and The National Nutrition Survey (The Ministry of Health, Labour and Welfare, Japan, 2000) by indirect human calorimeter, and the PAL of this study was 1.51±0.12 (Tanaka *et al*., 2003). The relatively higher proportion of the present subjects who participated in regular physical activity (more than twice a week and more than 30 min at a time) compared with the National Nutrition Survey (Ministry of Health, Labour and Welfare, Japan, 2006) is one of the potential reasons for higher PAL. However, the subjects with active exercise habits did not show significantly higher PAL compared with non-exercisers, though exercisers engaged in exercise 227±141 min/week on average. Schoeller *et al*. (1997) and Weinsier *et al*. (2002) suggested that a PAL of around 1.7 might be required to prevent weight regain in post-obese females. Brooks *et al*. (2004) also suggested that most adults maintaining a BMI in

Table 3 TEE and PAL among categories according to Dietary Reference Intake in Japan

		n	TEE (KJ/day)	PAL	P-value
I (light)	Mostly sedentary position doing reading, studying and talking, or sitting or lying position watching TV and listening to music with 1-h slow walk for walking and shopping	77	9.63±1.90	1.68±0.21	0.070
II (moderate)	Mostly sedentary position doing clerical work and housework with 2-h walk for commuting and shopping, and long hours of standing while meeting people doing housework	63	9.29±1.87	1.74±0.25	
III (light heavy)	In addition to moderate activity (II), 1 h of brisk walk, bicycle and other vigorous physical activity; mostly standing during farming, fishing with heavy musculer work for 1 h a day	6	9.64±2.04	1.85±0.31	
IV (heavy)	Engaged in heavy muscular work for about 1 h a day such as hard training, carrying lumbers, farming in the busy season and so on	4	12.31±1.21	1.91±0.30	

Abbreviations: PAL, physical activity level; TEE, total energy expenditure.
P-values were calculated by one-way analysis of variance for PAL.

Table 4 TEE and PAL among categories of International Physical Activity Questionnaire

Group	n	TEE (KJ/day)	PAL	P-value
Category 1 (insufficiently active)	82	9.49±1.90	1.70±0.24*	0.016
Category 2 (sufficiently active)	61	9.48±1.88	1.75±0.23	
Category 3 (highly active)	7	11.13±2.14	1.95±0.24	

Abbreviations: PAL, physical activity level; TEE, total energy expenditure.
*Significantly different from category III (highly active).
P-value was estimated by one-way analysis of variance for PAL.

the healthful range had PAL values >1.6. The higher proportion of subjects with lean to normal BMI (74%) in the present study might partly explain the relatively higher PAL in the present subjects.

In the public health status and epidemiological study, a simple questionnaire to assess the PAL is required. In the present study, we used the questionnaire in the DRI, Japan sixth edition and IPAQ. Highly active groups assessed both by DRI and IPAQ showed significantly higher PAL, though there were few subjects in these groups. In IPAQ, the highly active category consisted of subjects with 1500 met-min/week by vigorous activity or by a combination of walking, moderate or vigorous activities. In DRI, heavy is categorized as persons engaging in more than 1 h a day of muscular work. Among the healthy normal subjects in developed countries, vigorous physical activity could be easily assessed by questionnaire, and subjects who participated in these activities showed higher PAL compared to those with little or no vigorous physical activity.

There were no significant differences in PAL between light and moderate categories in DRI, or between insufficient active and sufficient active categories in IPAQ. There was a clear overlap of measured PAL in these lower two categories. The lower categories both by IPAQ and DRI are divided mainly by the duration of light to moderate physical activity. The duration of these activities is thought to pose more difficulty than vigorous activity in terms of response, and this made it difficult to categorize the less active population. However, the duration of these activities had much impact on PAL among subjects with the normal PAL range, because they spent an average 9% of their active time engaging in high-intensity activity, and the distribution of time spent in activities of low and moderate intensity determines the activity level (Westerterp, 2001).

In addition, we could not find any differences in PAL between exercisers and non-exercisers. In one study of weight reduction (Kempen *et al*., 1995), there were no significant differences in PAL and energy expended on physical activity between diet only and diet plus exercise treatment groups. This was considered the result of partial compensation in physical activity for the addition of training to dietary treatment during the non-exercise part of the day. It also suggests the importance of assessing non-exercise physical activity. Other recent studies also point out the importance of the proportion of light to moderate activity on TEE (Westerterp, 2003; Levine, 2004; Levine *et al*., 2005). In a future study, we should clarify the physical activity that has much effect on the TEE among sedentary to moderately active subjects, and the method of assessing accurately these physical activities.

One of the most important limitations of the present study is that BMR was predicted, not measured. Calculation of PAL using predicted BMR could lead to some error for individuals. This may have caused a wide variation in PAL among each category divided by sex and age groups or the questionnaire on physical activity. However, we thought the use of prediction equations for BMR would generate the present result. Many prediction equations are available for estimating BMR, but their applicability to other ethnic groups is uncertain (Hayter and Henry, 1993; Frankenfield *et al*., 2005). Ganpule *et al*. (2007) suggested recently that the use of FAO/WHO/UNU equations overestimated BMR among Japanese when compared with measured BMR. The predictive equations used in the present study were established based on the large database obtained under strictly controlled protocol, and have been reported to be accurate for Japanese (Taguchi *et al*., 2001; Rafamantanantsoa *et al*., 2003; Yamamura *et al*., 2003). Therefore, the error from using predicted BMR seems to be modest.

Another limitation is that subjects were not selected randomly from different activity levels. This caused unequal distribution of subjects across activity categories, which may have caused lower statistical power in comparison among activity categories.

In conclusion, the present study clarified the PAL among healthy normal Japanese and compared the PAL among the categories assessed by a simple questionnaire. In developed countries, highly active subjects seem to be easily assessed by a simple questionnaire. However, assessment of the PAL among sedentary to moderately active subjects is more complete, and must be addressed in a separate study.

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