Total Energy Expenditure of 16 Chinese Young Men Measured by the Doubly Labeled Water Method^{*}

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Abstract

Objective Doubly labeled water (DLW) method is the gold standard for measuring total energy expenditure (TEE). We used this method to measure TEE in Chinese young men.

Methods Sixteen healthy young men age 23 ± 1 years with body mass index 22.0 ± 1.4 kg/m² were recruited. TEE was measured by the DLW method, and basal energy expenditure (BEE) was determined by indirect calorimetry. We also conducted 24-h activity, energy balance and factorial approach to estimate energy requirements of the subjects.

Results TEE of subjects by DLW method was 9.45 ± 0.57 MJ/day (2258 ±180 kcal/day). The 24-h activity was 10.80 ±0.33 MJ/day (2582 ±136 kcal/day). The energy requirement, derived from energy balance observations, was 9.93 ± 1.32 MJ/day (2373 ±315 kcal/day). The BEE of 6.65 ± 0.28 MJ/day (1589 ±67 kcal/day), calculated by the adjusted Schofield equation, was significantly higher (P<0.001) than that measured by indirect calorimetry, 5.99 ± 0.66 MJ/day (1433 ±158 kcal/day). The TEE derived from the factorial approach was 10.31 ±0.43 MJ/day (2463 ±104 kcal/day).

Conclusion The TEE of Chinese young men measured by the DLW method was about 10% lower than the current recommended nutrient intake (RNI), suggesting that the RNI for Chinese men maybe overestimated. Further studies are warranted to determine the value of the estimated energy requirement.

Key words: Doubly labeled water; Total energy expenditure; Recommended nutrient intakes; Estimated energy requirement; Chinese young men

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INTRODUCTION

Based on reports from the 2002 Chinese National Health and Nutrition Survey, the average dietary energy intake of the Chinese people was 9420.6 kJ/day (2250 kcal/day) which is about 8% lower than that in 1992 (9741.6 kJ/day) and 10% lower than that in 1982 (10 423.6 kJ/day)^[1]. These data suggested that the energy intake of the Chinese decreased gradually.

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Conversely, the prevalence of chronic diseases related to energy intake imbalance-e.g., overweight, obesity, lipid disorders, hypertension, diabetes, cardiovascular disease-increased rapidly during those years. For example, the prevalence of overweight and obesity in those >18 years old in 2002 were 17.6% and 5.6%, respectively-about 38.6% and 80.6% higher, respectively, than those in 1992^[1]. Because social and of economic transformations, more Chinese tended to have more inactive lifestyles during these later years. It gives rise to a concern that the current diet energy-based recommended nutrient intake (RNI) (i.e., 2400 kcal/day for a man living at a light activity level) proposed by the Chinese Nutrition Society in 2000 may be overestimated^[2].

To establish the recommended estimated energy requirement (EER), an accurate measurement of the TEE of individuals who maintain an energy balance should be applied. This means that they should have not only energy intake equal to expenditure but should maintain a stable body weight.

Currently, the adjusted Schofield equation^[3] (5% lower than the original equation) is used to predict the basal energy expenditure (BEE), which depends on the physical activity level (PAL), to determine the energy requirement for the RNI for Chinese adults. The Schofield equation, however, was established using data that included values from а disproportionate number [3388/7173 (47%)] of Italian subjects. Many researchers pointed out that the Schofield equation might therefore overestimate the energy requirements for Chinese people by as much as 15%^[4].

Few studies have measured TEE of Chinese adults, with only sparse data available using doubly labeled water (DLW) measurements. Therefore, we assessed TEE in 16 young Chinese men using the DLW method. We compared various methods, including a factorial approach and energy balance records methods, with and physical DLW measurements to estimate the energy requirement of the subjects. We believe that the results from our study can contribute to future revision of the Chinese RNI standards.

SUBJECTS AND METHODS

Subjects

A total of 300 male students attending Bethune Military Medical College (Hebei province, China) were screened through a questionnaire. Inclusion criteria stated that the subjects should be healthy with no history of diabetes, thyroid disorder, or other metabolic disorder. They had to have a normal body mass index (BMI 18.5-24.0 kg/m²) and had maintained a stable weight for several months before the study. Subjects who had smoking, drinking, and/or bad eating habits, insomnia, and/or stress excluded. hematological were After and biochemistry examinations to rule out anemia, a thyroid disorder, and liver and kidney dysfunction, 16 eligible men, aged 20 to 26 years, were randomly selected.

The Ethical Review Committee of the National Institute for Nutrition and Food Safety, Chinese Center for Disease Control and Prevention approved this study. Written informed consent was obtained from all subjects.

The study was conducted for 16 days. During the study period, subjects were required to reside in a hotel at the college, and all food consumed by them was provided by the investigators. To maintain energy expenditure relatively constant throughout the study, subjects were required to follow their usual physical activities.

Total Energy Expenditure Measurements by DLW

For the first 2 days, the subjects moved into the hotel and got used to the environment and the foods. On the morning of the third day, immediately after collecting a predose urine sample, each subject was given a preweighed amount of DLW (Huayi Isotope, Changshu, China) that had been prepared and conditioned as a sterile solution in a sealed 125-mL plastic bottle. The bottles were weighed using an analytical balance to the nearest 0.1 mg (AX150; Mettler, Zurich, Switzerland) just before and after the DLW was consumed. The approximate doses were 160 mg H₂¹⁸O and 250 mg ²H₂O/kg body weight. Postdose urine samples were collected at 2, 4, 6, and 8 h and then once a day during the subsequent 13 days. The tap water used for cooking was also collected. Urine samples and tap water samples were put in 100-mL containers with airtight screw tops and then transferred in 5.0-mL cryogenic vials with screw tops. These vials were stored at -20 °C until analyzed.

All urine samples, tap water, and diluted DLW samples (diluted about 1000 times by the tap water collected from the field site) were analyzed at the Laboratory for Stable Isotope Geochemistry (Geological and Geophysical Research Institute, Chinese Academy of Sciences) with MAT-252 and MAT-253 (Thermo Finnigan; Thermo Electron, San Jose, CA USA) gas-isotope-ratio mass spectrometers for deuterium and 18 O, respectively. Enrichments of 2 H and 18 O were expressed as δ^2 H and δ^{18} O relative to Vienna Standard Mean Ocean Water (VSMOW).

Basal Energy Expenditure

The basal energy expenditure (BEE) of subjects was measured via indirect calorimetry with the K4b² portable metabolic system (Cosmed, Rome, Italy). With this procedure, after an overnight fast of 10-12 h, measurements were taken when subjects were fully awake but lying quietly and relaxed. Rates of oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured over 15 min using the K4b² apparatus. Data from the first 5 min were excluded, and the remaining data were averaged. Heart rate was simultaneously monitored to track the subject's anxiety level. BEE values were calculated from the VO₂ and VCO₂ using Weir's equation^[5]. The Cosmed K4b² was calibrated prior to each test following the manufacturer's guidelines.

Anthropometric Measurements and Body Composition

Anthropometric measurements were made every day in the morning. While the subjects stood barefoot, weight with precision to 0.01 kg was measured using a digital electronic scale (HW100KGL; Scaleman.com) and height with precision to 0.1 cm using a stadiometer. Body composition was determined using a four-terminal bioelectric impedance analyzer (101; RJL Systems, Clinton Township, MI, USA) as described by Lukaski et al.^[6]. Fat-free mass (FFM) was calculated using the Deurenberg equation^[7].

Energy Requirement by the Balance Method

A 3-day rotating menu was used during the 16-day experiments. The provided diets mimicked a typical Chinese diet. The diets' compositions were designed according to the food categories and nutrient intake of the Chinese population from data found in the 2002 Chinese National Health and Nutrition Survey. The energy sources derived from the carbohydrate, fat, and protein were in a ratio of approximately 11:6:3. The allocations at breakfast, lunch, and dinner were 5:8:7. For each meal, foods were weighed before they were given to the subject. Leftovers were also weighed. Thus, we were able to quantify the amounts of each food item that the individual subject consumed. Each diet sample was stored for measuring the contents of total protein, total fat, water, and ash to determine its total energy. Then, according to the change of body weight during the experiment, the energy requirement was calculated using the following equations (where d = days)^[8].

Energy requirement (MJ/d) = energy intake (MJ/d) + decrease of body weight (kg) × 29 ÷ experimental period (days) (1) or

Energy requirement (MJ/d) = energy intake (MJ/d)-increment of body weight $(kg) \times 29 \div$ experiment period (days) (2)

Physical Activity Records

According to the participants' lifestyle, a 24-h activity form was developed that included all of the possible activities in which the subject might participate. The subjects were trained to fill the form daily, recording their activity types and the time devoted to them. The records were completed for the 14 experimental days. The administrator checked the records every day and verified their existence. The total time for all of the activities 1440 min/day^[9]. should equal The energy expenditure value of the subject was calculated as the sum of the intensity of each reported activity [in metabolic equivalents (METs)/min]^[10] multiplied by the activity's duration (in minutes) and the unit of the MET (0.0175 kcal·kg⁻¹·min⁻¹) and body weight of the subject.

Physical Activity Level

The physical activity level (PAL) was calculated by dividing TEE (measured by DLW) by BEE (measured by the $k4b^2$).

Calculation for TEE

The TEE for every subject was calculated using a multi-point protocol proposed by International Atomic Energy Agency (IAEA) guidelines^[11].

First, convert the $\delta^2 H$ and $\delta^{18} O$ into atom abundance (ppm²H and ppm¹⁸O) using the following equations.

ppm ²H = 1 000 000/{1 + $[1/(\delta^2 H/1000 + 1) \times 0.000 155 76]}$ (3)

ppm 18 O = 1 000 000/{1 + [1/(δ^{18} O/1000 + 1) × 0.002 005]} (4)

Second, the enrichments ppm xs 2 H and ppm xs 18 O (xs = excess) of postdose urine samples were



Figure 1. Elimination regression curve of ²H and ¹⁸O in a subject throughout the experiment.

Third, the isotope dilution spaces (N_D and N_O), where D and O are deuterium and ¹⁸O, respectively were calculated using the following equation.

Nx = $[(W \times A/a) \times (\Delta DD/\Delta BW)]/1000$ (5) Where x is ²H or ¹⁸O; W is the amount of water used to make the diluted dose; a is the amount of the dose used in this dilution; A is the dose taken by the subject; ΔDD is the enrichment measured in the diluted dose; ΔBW is the anti-log of the y intercept of the graph of Ln ppm xs ¹⁸O or Ln ppm xs ²H versus time.

Fourth, the average total body water (TBWavg) was calculated by the following equation:

TBWavg (mol) = $(No/1.007 + N_D/1.041)/2 \times 1000/18.0153$ (6)

Fifth, carbon dioxide production (rCO_2) was calculated from TBWavg (mol) and the isotope elimination rate (Ko and K_D were the gradients of the plot of Ln ppm xs ¹⁸O or Ln ppm xs ²H versus time, respectively) as:

 $rCO_2(L/day) = 0.455 \times TBWavg \times \{[1.007 \times (-K_0)] - [1.041 \times (-K_D)]\}$ (7)

Sixth, the food quotient (FQ) of the participant was calculated using the total protein, fat, and carbohydrate intakes during the experimental period^[12].

 $FQ = 0.8 \times P_p + 0.71 \times P_f + 1 \times P_c$ (8)

Where Pp, Pf, and Pc are the percentage energy from protein, fat, and carbohydrate, respectively.

Finally, the TEE was calculated using the modified Weir's equation:

 $TEE(kcal/day) = rCO_2(L/d) \times (1.10 + 3.90/FQ)$ (9)

Energy Requirement by Factorial Approach

The energy requirement (ER) was calculated by

the equation

$FR = nBFF \times PAI$	(10)
	(10)

Where pBEE is the predicted BEE using the revised Schofield equation

 $(15.3 \text{ w} + 679) \times 95\%$ (kcal) (11)

Where w is the body weight in kilograms. The PAL value is 1.55, corresponding to the light physical activity level of Chinese adult men, as proposed by the Chinese Nutrition Society in 2000. Because most PALs of the subjects were within the range of light physical activity level, it was chosen to predict the energy requirement of the subjects.

Data Analysis

Statistical analysis was performed using the SPSS software package for MS Windows (release 20.0; SPSS, Chicago, IL, USA) and Microsoft Excel 2003. Descriptive statistics (mean±SD) were presented. The paired test was used to assess the statistical significant differences between measurements. All statistical tests were two-sided hypothesis tests performed at the 5% level of significance.

RESULTS

Characteristics of the Study Participants

The characteristics of the study participants are presented in Table 1. The mean BMI value of the subjects was 21.97 kg/m², and the mean fat-free mass was 54.10 kg.

Table 1. Characteristics of the Study Participants

Characteristic	Value
Age (years)	23±1
Height (cm)	172.0±2.7
Body weight (kg)	64.93±4.59
BMI (kg/m²)	21.97±1.35
Fat-free mass (kg)	54.10±3.50
Lipid mass (%)	15.27±3.67

Energy Expenditure and Physical Activity Level

The TEEs measured by DLW, BEEs measured by portable indirect calorimetry, and predicted BEEs calculated by the revised Schofield equation are presented in Table 2. The average TEE measured by DLW was 9.45 ± 0.75 MJ/d. The calculated BEE derived by revised Schofield equation was significantly higher than the measured BEE (6.65± 0.28 MJ/day vs. 5.99 ± 0.66 MJ/day, *P*<0.001). The average PAL was 1.59 ± 0.22 .

Energy Requirement by Energy Balance Method

The macro-nutrients and energy intakes of each subject are presented in Table 3. The average energy

intake was 9.99±0.55 MJ/day (2387±132 kcal/day). According to the change in body weight, the average energy requirement was 9.93±1.32 MJ/day (2373±315 kcal/day).

Subject No.	TE	TEE		BEE		pBEE ^a		DAI ^b
Subject NO.	MJ/d	kcal/d	MJ/d	kcal/d	MJ/d	kcal/d	FAL	
1	8.30	1984	6.11	1459	6.57	1571	1.36	Ī
2	9.51	2273	6.15	1469	6.99	1671	1.55	
3	8.34	1994	6.30	1505	6.90	1649	1.32	
4	8.37	2000	5.34	1275	6.89	1647	1.57	
5	10.66	2547	5.50	1315	6.52	1558	1.94	
6	9.08	2170	5.37	1284	6.41	1532	1.69	
7	9.05	2162	6.62	1582	6.42	1533	1.37	
8	10.39	2484	4.90	1171	6.64	1586	2.12	
9	9.24	2208	6.68	1597	6.93	1657	1.38	
10	9.50	2270	6.15	1471	6.38	1526	1.54	
11	10.01	2393	7.01	1676	7.07	1689	1.43	
12	9.48	2265	6.10	1457	6.90	1649	1.55	
13	10.14	2423	6.93	1657	6.30	1506	1.46	
14	9.81	2344	5.73	1369	6.19	1480	1.71	
15	8.89	2124	4.88	1167	6.44	1540	1.82	
16	10.39	2483	6.14	1467	6.84	1634	1.69	
Mean	9.45	2258	5.99	1433	6.65	1589	1.59	
SD	0.75	180	0.66	158	0.28	67	0.22	

Table 2. Total Energy Expenditure, Basal Metabolic Rate, and PAL of the Participants

Note. ^aCalculated by the revised Schofield equation: $(15.3 \text{ w} + 679) \times 95\%$ (kcal), where w is body weight (kg). The pBEE is significantly higher than the measured BEE (*P*<0.001). ^bRatio of TEE to measured BEE. Abbreviation. TEE = total energy expenditure; BEE = basal metabolic rate; pBEE = predicted BEE; PAL = physical activity level.

Table 3. Energy Requirements of the Participants, Determined by the Energy Balance Method

Subject	Protein Intake	Fat Intake	CHO Intake	Fiber Intake	Energy Intake		Change in BW over 14 Days	V Energy Requirement	
NO.	(g/d)	(g/d)	(g/d)	(g/d)	MJ/d	kcal/d	(kg)	MJ/d	kcal/d
1	92±13	42±7	334±36	33±3	8.99	2148	-0.28	9.57	2287
2	90±31	40±14	374±34	48±5	9.67	2312	-0.28	10.25	2451
3	97±7	43±7	387±32	24±4	9.92	2371	-0.14	10.21	2440
4	101±11	42±7	409±46	44±4	10.49	2506	-0.51	11.54	2758
5	101±11	45±7	384±34	32±3	10.08	2409	1.01	7.99	1909
6	98±9	45±8	357±30	45±7	9.69	2315	0.73	8.17	1954
7	103±10	45±8	406±42	48±3	10.61	2537	0.61	9.35	2235
8	98±11	41±7	363±36	32±3	9.53	2277	1.02	7.41	1772
9	106±11	46±7	423±46	29±4	10.83	2588	-0.71	12.30	2940
10	106±9	46±6	421±27	27±5	10.78	2576	0.61	9.51	2274
11	96±9	47±9	361±60	26±4	9.64	2303	-0.34	10.34	2471
12	97±7	44±6	362±22	48±6	9.74	2328	-0.62	11.02	2635
13	94±11	44±6	342±22	34±3	9.24	2208	-0.48	10.23	2446
14	101±7	44±7	412±43	46±3	10.63	2540	0.54	9.51	2273
15	98±9	43±8	384±33	31±4	9.95	2377	-0.04	10.03	2397
16	101±9	47±7	374±33	37±5	10.03	2397	-0.68	11.44	2734
Mean±SD	99±5	44±2	381±28	36±8	9.99±0.55	2387±132	0.03±0.62	9.93±1.32	2373±315

Estimated Energy Requirements by Different Methods

The estimated energy requirements (EERs) of subjects by different assessment methods are showed in Table 4. Among the methods, from high to low, the values of EERs were 10.80±0.57 MJ/day (by physical activity records), 10.31±0.43 MJ/day (by the factorial approach), 9.93±1.32 MJ/day (by energy balance studies), and 9.45±0.75 MJ/day (by the DLW method). The results of other methods were all significantly higher than those obtained with the DLW method.

Table 4. Estimated Energy Requirements of theParticipants by Different Assessment Methods

Method	Estimated Energy Requirement			
	MJ/d	kcal/d		
Doubly labeled water (DLW)	9.45±0.75	2258±180		
Energy balance ^a	9.93±1.32	2373±315		
Factorial approach ^b	10.31±0.43	2463±104		
Physical record ^c	10.80±0.57	2582±136		

Note. ^aSignificantly higher than the DLW method (P=0.04); ^bSignificantly higher than the DLW method (P=0.002); ^cSignificantly higher than the DLW method (P<0.001).

DISCUSSION

The DLW method has come to be considered the most reliable measurement of TEE for free living people. With the DLW data accumulating for a wide range of age groups and body sizes, the technique has also been widely used for determining population EERs in Western countries^[13-14].

So far, there are only three publications regarding TEEs of Chinese populations measured by the DLW method^[15-17]. Jiang et al. investigated the energy requirement of infants^[15]. Liu et al., who is in our group, estimated the energy requirement for young Chinese women^[16]. Yao et al. reported that the average TEE for urban Chinese men was 12.1±0.3 MJ/day, which was a much higher value than what we found in our study ^[17]. It must be noted, however, that the average body weight and PALs of subjects in the study by Yao et al. were higher than those in ours: 73.43±2.24 vs. 64.93±4.59 kg for body weight and 1.77±0.04 vs. 1.59±0.22 for PALs, respectively. For comparison, the data were adjusted using the reference body weight (63 kg) of Chinese

men ages 18-50 years with different physical activities, as proposed by the Chinese Nutrition Society in 2000^[2]. After adjusting for body weight and PALs, the data in Yao et al.'s study showed that the EERs of Chinese men were 9.09 MJ/day (2172 kcal/day), 10.44 MJ/day (2495 kcal/day), and 12.32 MJ/day (2944 kcal/day) for light (PAL 1.55), moderate (PAL 1.78), and heavy (PAL 2.10) activity levels, respectively. In our study, the adjusted EERs of Chinese men were 8.94 MJ/day (2136 kcal/day), 10.26 MJ/day (2453 kcal/day), and 12.11 MJ/day (2894 kcal/day) for light, moderate, and heavy activity levels, respectively. These adjusted DLW data were all lower than the current RNI for different PALs of Chinese adult men (Figure 2). It indicates that the recommended energy requirements might be overestimated about 10% for adult Chinese men of different PALs. The study conducted by Liu et al. also indicated that the current RNI for Chinese young adult women was overestimated by about 10%^[17]. From studies using the DLW technique, the current RNI for different PALs might be overestimated for Chinese adults.



Figure 2. Comparison of adjusted estimated energy requirements (EER) derived from the doubly labeled water (DLW) technique with the recommended nutrient intake (RNI) of Chinese men of different PALs. Adjusted 1: adjusted data from Yao et al.^[17]. Adjusted 2: adjusted data from the present study.

When data obtained with the DLW method are not available, BEE is an important index for estimating the energy requirement by the factorial approach^[18]. The current RNI for Chinese adults is based on the factorial method where the BEE is estimated using the adjusted Schofield equation. The results of the present study showed that the BEE measured by indirect calorimetry was significant lower than the BEE predicted from the adjusted Schofield equation. The pBEE was overestimated by about 9%, which might be the reason the current RNI for Chinese adults is overestimated. Other studies^[19-20] have also shown that the Schofield equation overestimated the BEE of Chinese adults and suggested it might not be suitable for a Chinese population. These studies developed new prediction equations of BEE for Chinese adults, but whether these equations are adequate requires further investigation.

The value of the recommended energy requirement is important for the health of both the individual and the overall population. Even when the energy intake is higher than the energy requirement by only several kilocalories, accumulation for a relative long time causes overweight, obesity, and related chronic diseases such as hypertension, diabetes, and cardiovascular disease, among others^[21]. Hence, revising the current RNI for Chinese adults should be taken into consideration.

We also used the energy balance and physical record methods to estimate the EER of the participants. The results demonstrated that both of these methods significantly overestimated the EER for Chinese young men. Among the methods predicting the EER, the results of the energy balance studies are closest to those achieved with the DLW study. Another study investigating the energy requirements of Chinese young women^[16] found that the results of energy balance and DLW studies are similar (1832±96 *vs.* 1830±118 kcal/day). It suggested that if DLW data are not available because of the high cost or lack of equipment, the energy balance method may be a good choice for obtaining ER data.

The objective of the present study was to provide useful information by collecting data from a group of young Chinese men using the DLW method. Although the study was conducted under carefully controlled conditions, it does have some limitations. For example, the participants are from a narrow range of age and occupation, and the sample size was small. Further studies on a larger scale and wider range age and occupations should be conducted using the DLW method to assess the EER of Chinese populations.

In conclusion, the present study showed that the energy requirements of young adult Chinese men as determined with the DLW method were about 10% lower than the current RNI proposed by the Chinese Nutrition Society in 2000. In the primary analysis, the results are consistent with those of another DLW study. Considering the results of the 2002 Chinese National Health and Nutrition Survey, the RNI value for Chinese adult men might need to be reduced based on the DLW data. More investigations are needed to confirm our findings.

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