Short Communication

Dietary energy requirements of young adult women in China by the doubly labeled water method

Jian-Min Liu PhD[1,2], Xiao-Guang Yang PhD[1], Jian-Hua Piao BSc[1], Rui Sun PhD[1], Ying Tian PhD[1]

[1]*Key Laboratory of Trace Element Nutrition MOH, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, China*
[2]*Hebei Medical University, Shijiazhuang, China*

Objective: The current recommendations on dietary energy requirements in China have been based on total energy expenditure (TEE) derived from theoretical factorial calculations expressed as multiples of basal energy expenditure (BEE). Few direct measurements of TEE and BEE have been conducted in Chinese peop1e. This study was designed to measure the TEE and BEE of healthy, light active women of normal body weight living in China, and to evaluate the recommendations on dietary energy requirements by comparing the data correspondingly. Design: 16 healthy female adults aged 22.1±1.2 y with a body mass index (in kg/m^2) of 20.4±1.7 were selected. Under well-controlled conditions, TEEs were determined by using the doubly labeled water method. At the same time, BEEs were measured by using indirect calorimetry. Results: Measured TEE was 1380±118 kcal/d, which was close to the value of energy intake (1382±96 kcal/d). Comparing the measured TEE with the recommended (Chinese recommended value for this group is 2100 kcal/d), the recommended was substantially higher than the measured by 15% or so. Meanwhile, the calculated BEE (1230±65 kcal/d) by using the adjusted Schofield equation proposed by the Chinese Nutrition Society was significantly higher (p <0.001) than the measured BEE (1130±110 kcal/d), by 9% or so. Conclusion: Current recommended energy intake for young adult women may need to be revised on the basis of measured TEE and BEE.

Key Words: energy requirement, Chinese, doubly labeled water, basal energy expenditure, total energy expenditure

INTRODUCTION

Recommendations for energy requirements provide information on the dietary energy requirements of different populations. It is important that the recommendations be accurate because they provide standards for optimal nutrition and might be used as the basis for determining the need for nutrition-intervention programs in less-developed countries.[1] Currently, most developing and transitional countries (including China) still use the factorial method recommend by the 1985 FAO/WHO/UNU report for estimating individual and population needs of energy.[2,3] However, concerns have been raised about the accuracy of the WHO estimates because they are based on theoretical factorial calculations of energy needs rather than on direct measurements of total energy expenditure (TEE) in individuals leading different lifestyles.[4-8] The WHO expert consultative panel also suggested that future guidelines should be based on measurements of energy expenditure when available.

The doubly labeled water (DLW) method is considered a criterion method for estimating energy expenditure (EE) in free-living persons. A lot of researchers have used the technique to re-evaluate the usual energy needs of different groups.[9] Although there is now a considerable body of doubly labeled water data from individuals living in Western countries, the cost of isotopes and analyses and the requirement for an isotope ratio mass spectrometer prohibits the method from being used in developing countries. To our knowledge, there is still relatively little information on individuals living in developing countries,[10] and within China, minimal data are available.

The present study was initiated to obtain information on the energy requirements of Chinese adults calculated from energy expenditures by using the DLW method, and compare the energy values with the recommended.[3] A group of healthy, weight-maintaining Chinese young adult women were chosen as the subjects. We have also measured their basal energy expenditures (BEEs), which was an important factor in the theoretical factorial calculations for the determination of energy requirements proposed by the WHO.

MATERIALS AND METHODS

Subjects

The study was conducted at the Bethune Military Medical

Corresponding Author: Dr Xiao-Guang Yang, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, 29 Nanwei Road, Beijing, China, 100050.
Tel: +8610-83132798; Fax: +8610-83132808
Email: xgyangcdc@gmail.com
Manuscript received 28 February 2010. Initial review completed 17 June 2010. Revision accepted 27 July 2010.

College. The volunteers were recruited from the students of the college. Through questionnaire and normal physical and routine blood examinations, sixteen healthy female students were selected as the subjects for participation in this study. Subjects were 20-25 years old, healthy, with no history of diabetes or any other metabolic disorder and were not in their menstrual periods during the study. They all had normal body weights (body mass index, BMI, in kg/m^2 of 18.5-24) and had maintained stable weights for several months before the study. The nature and purpose of the study were explained to each subject and written informed consents were obtained from them. The study protocol was approved by the Human Studies Ethics Committee of the Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention.

Experimental protocol

The investigation was conducted over a 16-d period. During the study, the participants were required to reside at the hostel of the college and all food consumed by them was provided by the investigators. In order to keep energy expenditure relatively constant throughout the study, subjects were required to follow sedentary or light-activity lifestyles.

The first two days was the adaptation period, the subjects moved into the hostel to get used to the environment. The test period was 14-d. TEE was measured throughout the 14 days by using the doubly labeled water technique. At the same time, energy intake, basal metabolic energy expenditure and some other measurements were made as described below.

Anthropometric measurements

Anthropometric measurements (including weight and height) were obtained daily. After an overnight fast, weight was measured to 0.01 kg by using a digital electronic balance (HW100KGL, Japan) in the morning with the subjects wearing minimal clothing, and height was measured to 0.1cm with a stadiometer while subjects stood barefoot.

Body composition was determined by using a four-terminal bioelectric impedance analyzer (101, RJL Systems, USA) as described by Lukaski *et al.*[11] and fat-free mass (FFM) was calculated from the equation proposed by the Chinese Nutrition Society.[3]

Physical activity level

To evaluate the physical activity levels of the participants, a 24-h activity record was administered to the subjects. Habitual activities carried out during the entire 14-d study were recorded by each subject on a time sheet and each record was checked daily for completeness and clarity by the investigators. Categorized the activities and counting up the time engaged in motionless (sitting or standing still, except sleeping) or motion (standing activities) respectively, we could assess the physical activity levels of the participants as such. At the same time, the subjects were required to wear a pedometer (PE828, Oregon, USA) all day long during the test, except when sleeping or showering. From the data (steps per day) obtained from the pedometer, we could also get a preliminary assessment of their physical activity level.

Measurements of basal energy expenditure (BEE)

Basal energy expenditures of subjects were measured via the recently developed $K4b^2$ portable metabolic system (Cosmed, S.R.L., Rome, Italy) as detailed elsewhere.[12] In this procedure, after an overnight fast of 10-12 hours, the subjects were gently awakened from sleeping and lying quietly in bed. They were instructed to relax and avoid hyperventilation, fidgeting and sleeping during measurements. Rates of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured over 15min by using $K4b^2$ equipment. Data from the first 5 min were excluded and the remaining data were averaged. Values for BEE were calculated from VO_2 and VCO_2 by using Weir's equation.[13] Calibration of the Cosmed $K4b^2$ was conducted prior to each test following the manufacturer's guidelines.[14]

Energy intake

During the 14-d study, the subjects ate their three meals under the supervision of the research staff and energy intakes were adjusted during the first few days until their weight stabilized. The provided diets mimicked a typical Chinese diet, approximately an average of 55%-65% of the energy was derived from carbohydrate and 20%-30% from fat, with a food quotient of 0.88. By weighing what was given and leftover, we could quantify amounts of each food item consumed. At the same time, samples of each food item were collected for composition analysis. Energy content was determined on the basis of the Atwater constants.

Energy expenditure (doubly labeled water)

The EE over the 14-d study was determined by using the multi-point DLW method. On the first day of the experiment, the subjects got up at 6:00 in the morning. After providing a baseline urine sample, each participant ingested a known quantity of doubly labeled water dose (Huayi Isotope Co, Changshu, China), which were prepared and conditioned as sterile, apyrogenic solutions in a sealed 100-mL plastic bottle, to provide 150 mg $H_2{}^{18}O$/kg body weight and 250 mg ^2H_2O/kg body weight. Urine samples were collected five times on day 1, once before administration of the DLW and then 2, 4, 6 and 8 hours after administration of the dose. In the subsequent days of the protocol, urine samples were collected once a day. Urine samples were stored in several 5 mL sealed sample tubes at -20 °C. Isotopic enrichment of urine samples were analyzed at the Laboratory for Stable Isotope Geochemistry (Geological and Geophysical Research Institute, Chinese Academy of Sciences) with a MAT-252 and MAT-253 (Thermo Finnigan, USA) gas-isotope-ratio mass spectrometer for deuterium and ^{18}O respectively. Enrichments of ^2H and ^{18}O were expressed as δ^2H and $\delta^{18}O$ relative to V-SMOW. After natural logarithmic transformation of these data, ^{18}O and ^2H were plotted against time. The pool sizes (N_D and N_O, where D and O are deuterium and ^{18}O) were calculated as the reciprocal of their respective intercepts and the fractional turnover rates (k_D and k_O) from the slopes of their respective disappearance curves. Fractional turnover rates for each isotope and their dilution spaces were computed and used to compute CO_2 expiration rate (rCO_2).[15-17] Values for TEE were calculated from rCO_2 by using Weir's equation.

Statistical analyses

Data were analyzed by the SPSS software (version 10.0; SPSS, Inc, Chicago, USA). Descriptive data were presented as mean±SD. The Wilcoxon signed rank test for paired variables was used to ascertain significant differences between measurements. For all measurements, results were considered statistically significant at $p < 0.05$.

RESULTS

Anthropometric measurements

Characteristics of the participants are presented in Table 1. Sixteen healthy individuals (female, aged 22.1±1.2 y) were recruited for this study. The mean BMI was 20.4±1.7. Body weights and compositions (expressed as fat-free mass) were monitored daily and the result showed that the changes were not significantly different from zero (Table 1).

Physical activity level (PAL)

The average number of steps per day accrued via pedometry was 9630 steps/d (Table 1). According to the proposal of Tudor-Locke C about pedometer-determined physical activity in healthy adults,[18] our subjects might be considered as "somewhat active". Table 1 also presents the average time spent in motionless or motion activities on each study day and its relative percentage. According to the physical activity level classification for Chinese adults proposed by the Chinese Nutrition Society,[3] our subjects appeared to be appropriately classified as performing light to middle active work on average.

Energy intake (EI)

Average level for daily macronutrient intakes and energy supplied are presented in Table 2. Total daily energy intake (EI) was 1832±96 kcal/d. Approximately an average of 54% of the energy was derived from carbohydrate and 30% from fat.

Basal energy expenditure (BEE)

As presented in Table 3, measured BEE was 1130±110 kcal/d, whereas the calculated BEE was 1230±65 kcal/d. The measured BEE was significantly lower (p=0.0021) than the value for BEE calculated from body weight by using the adjusted Schofield equation for women aged 18-44 y proposed by the Chinese Nutrition Society.[3,19] The calculated BEE overestimates BEE about 9%.

Total energy expenditure (TEE)

As presented in Table 3, the average energy expenditure by using the DLW method was 1830±118 kcal/d.

Table 1. Characteristics of the study participants

Characteristics	Value
Age (y)	22.1±1.2
Height (cm)	164±3.2
BMI (kg/m^2)	20.4±1.7
Body weight (kg)	54.5±4.7 (54.4±4.8, 54.5±4.6)†
Fat-free mass (kg)	41.3±2.3 (40.8±2.6, 41.4±2.4)†
Time devoted to motionless activities (min) ‡	705±39 (72±4%)
Time devoted to motion activities (min) ‡	272±35 (28 ±4%)
Pedometer (steps/d§	9630±1010

Values are expressed as mean ± SD, n = 16.
†data at the beginning and end of the study in parentheses;
‡Time devoted to motion or motionless activities derived from the time-motion record, percentage in parentheses;
§Average steps per day obtained from the pedometer.
BMI= body mass index

Table 2. Macronutrient and energy intakes of the subjects

Variable†	Value
Carbohydrate (g/d)	246±13.3 (53.7±1.4)
Protein (g/d)	66.0±4.7 (14.4±0.5)
Fat (g/d)	65.1± 4.4 (32.0±1.2)
Energy intake (kcal/d)	1832±96

Values are expressed as mean ± SD, n = 16.
†Macronutrient intakes calculated from amounts of each food item consumed and its nutrient compositions analyzed in the lab. Energy intake was determined on the basis of the Atwater constants (9 kcal/g fat, 4 kcal/g carbohydrates, and 4 kcal/g protein), percentage of energy supply in parentheses.

Table 3. Total energy expenditure determined by using the doubly labeled water method, energy intake and basal energy expenditure in young women

Variable	Value
Total energy expenditure (TEE, kcal/d)	1830±118
Energy intake (EI, kcal/d)	1832±96
Measured BEE (kcal/d) †	1130±110
Calculated BEE (kcal/d) ‡	1230±65**
TEE/BEE§	1.62±0.14

Values are expressed as mean ± SD, n = 16.
†Measured BEE, basal energy expenditure measured via K4b^2 portable metabolic system;
‡Calculated BEE by the adjusted Schofield equation proposed by the Chinese Nutrition Society: $BEE(kcal/d) = (14.7W + 496) \times 95\%$ (W-body weight, kg);
§TEE/BEE, the ratio of TEE to Measured BEE.
**Significant differences between measured and calculated BEE, p <0.01

DISCUSSION

The cost of isotopes and analyses for the doubly labelled water method is high, about US $1,500 per person in our study. In order to ensure the accuracy and representative of the experimental results with limited resources, we have used the nutrition concept of "standard person". This standardization applies to the participants and their living conditions, their physical activity levels and there three meals everyday as described below.

Firstly, all subjects were selected from a population who had participated in a basic metabolic measurement program that our team conducted previously. The familiarity with the researchers and equipments facilitated the smooth conduction of the study. And the average body weight (54.5 kg) of the subjects was very close to the medium value (55.0 kg) for 18-44 y female inhabitants obtained from the last Chinese Nutritional Survey in 2002.

Secondly, the study was conducted under controlled feeding conditions. By comparing the average level for

the daily macronutrient intakes with the dietary consumption level of this area obtained from the last Chinese Nutritional Survey in 2002, we could concluded that the test food consumption patterns appeared typical of this area.

Thirdly, the physical activity levels of the subjects were monitored. PAL, as defined by James et al,[20,21] is equal to TEE divided by BEE. Therefore, we calculated the PAL as being equal to the measured TEE divided by the measured BEE. The PAL ranged from 1.41 to 1.83 in individual subjects, with a mean of 1.62 (Table 3). According to the physical activity level classification for Chinese adults proposed by the Chinese Nutrition Society,[3] the mean PAL of 1.62 indicated that our subjects were performing light to moderate levels of activities. This is consistent with the result obtained from pedometry method (9630 steps/d) and time-motion method (72% motionless) as described previously. Briefly, our subjects appeared to be appropriately classified as performing light to middle active work on average.

So under well-controlled conditions DLW technique was used to measure the TEEs over 14d in 16 healthy young women of normal body weight who were performing light to middle active work. The mean value for TEE was 1830 kcal/d, which was near to the value of EI as presented in Table 3. This similarity between TEE and EI is consistent with the observation of approximately constant body weight in the group taken as a whole and confirms the accuracy of the TEE estimations. These values are lower than the current recommendations for energy requirements (2100 kcal/d) in young women with a similar lifestyle. The RNI estimates of energy needs are considerably higher than the measured TEE values, overestimate by 15% or so.

Some validation works of the factorial method against a criterion method such as DLW for determining EE had been made in the past few years, but the results were quite different. Some demonstrated that the factorial method underestimated EE in free-living conditions, while the others got the results of the opposite. For example, in two direct comparisons of factorial energy requirement estimates with DLW, one confirmed that the factorial method underestimated energy needs,[22] while the other found no difference between the methods in an elderly population with a mean age of 70 years.[23] A comparison of the factorial energy requirement estimates with whole-body indirect calorimetry measurements in Canadian adults showed that the FAO/WHO/UNU (1985) procedures may overestimate daily energy needs, particularly in sedentary individuals.[24] Our results appear to be consistent with that.

The reason for the overestimation requires investigation but it may be due to the overestimation for BEE. BEE is a major determinant of 24-h EE. The FAO/WHO/UNU report expressed the energy requirements of groups and individuals based on a factorial method (expressed as multiples of BEE), while in the present study we measured the BEEs of individuals to reduce errors from incorrect predictions from published equations proposed by the FAO/WHO/UNU report. As presented in Table 3, the calculated BEE overestimates BEE by about 10%. A 10% overestimation of BEE may results in a 10% overestimation of TEE and that may result in the overestimation for RNI.

This study may provide some useful experience in the data collection of TEE by using the doubly labeled water method from individuals living in China. Although this study was conducted under carefully controlled conditions, it does have some limitations. The participants comprised of a convenience sample and were not necessarily representative of the normal Chinese population. Further studies are needed to determine whether this overestimation also occurs in other population and age groups.

ACKNOWLEDGEMENTS

This study was supported by the National Natural Science Foundation of China (Grant No. 30771811) and the National Technology R&D Program in the 11th Five year Plan of China (Grant No. 2008BAI58B01). The authors would like to acknowledge the volunteers for their participation in this research.

AUTHOR DISCLOSURES

All authors have approved the manuscript. We declare that we have no conflict of interest in connection with this paper.

REFERENCES

1. Roberts SB, Heyman MB, Evans WJ, Fuss P, Tsay R, Young VR, William JE. Dietary energy requirements of young adult men, determined by using the doubly labeled water method. Am J Clin Nutr. 1991;54:499-505.
2. FAO/WHO/UNU. Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation. Technical Report Series, 724. Geneva: World Health Organization; 1985.
3. Chinese Nutrition Expert Panel. Chinese dietary reference intakes. Beijing: Chinese Light Industry Publishing House; 2000.
4. Prentice AM, Black AE, Coward WA, Davies HL, Goldberg GR, Murgatroyd PR, Ashford J, Sawyer M, Whitehead RG. High levels of energy expenditure in obese women. Br Med J. 1986;292:983-7.
5. Goran MI, Poehlman ET. Total energy expenditure and energy requirements in healthy elderly persons. Metabolism. 1992;41:744-53.
6. Reilly JJ, Lord A, Bunker VW, Prentice AM, Coward WA, Thomas AJ, Briggs RS. Energy balance in healthy elderly women. Br J Nutr. 1993;69:21-7.
7. Jiang ZQ, Yan Q, Su YX, Acheson KJ, Thélin A, Piguet-Welsch C, Ritz P, Ho ZC. Energy expenditure of Chinese infants in Guangdong Province, south China, determined with use of the doubly labeled water method. Am J Clin Nutr. 1998;67:1256-64.
8. Yao M, McCrory MA, Ma G, Li Y, Dolnikowsk GG, Roberts SB. Energy requirements of urban Chinese adults with manual or sedentary occupations, determined using the doubly labeled water method. Eur J Clin Nutr.2002;56:575-84.
9. Black AE, Coward WA, Cole TJ, Prentice AM. Human energy expenditure in affluent societies: an analysis of 574 doubly labeled water measurements. Eur J Clin Nutr. 1996; 50:72-92.
10. Chen YJ, Wang XS. The application of doubly labeled water technique in the assessment of energy metabolism. Chin J Sports Med. 2005;24:119-24. (In Chinese).
11. Lukaski HC, Bolonchuk WW, Hall CB, Siders WA. Validation of tetrapolar bioelectrical impedance method to assess human body composition. J Appl Physiol. l986;60:1327-32.
12. Pinnington HC, Wong P, Tay J, Green D, Dawson B. The level of accuracy and agreement in measures of FEO2, FECO2 and VE between the Cosmed K4b2 portable, respi-

ratory gas analysis system and a metabolic Cart. J Med Sci Sports. 2001;4:324-35.
13. Weir JB. New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol. 1949;109: 1-9.
14. Cosmed SRL. K4b2 User Manual. Rome: Cosmed SRL; 1998.
15. Coward WA. The doubly-labeled water (2H218O) method: principles and practice. Proc Nutr Soc. 1988;47:209-18.
16. Speakman JR, Nair KS, Goran MI. Revised equations for calculating CO2 production from doubly labeled water in humans. Am J Physiol. 1993;264:E912-7.
17. Westerterp KR. Food quotient, respiratory quotient, and energy balance. Am J Clin Nutr. 1993;57:759S-65.
18. Tudor-Locke C, Bassett DRJ. How many steps/day are enough? Preliminary pedometer indices for public health. Sports Med. 2004;34:1-8.
19. Schofield W, Schofield C, James WPT. Basal metabolic rate-review and prediction together with an annotated bibliography of source material. Hum Nutr Clin Nutr. 1985;39C: 1-96.
20. James WPT, Ferro-Luzzi A, Waterlow JC. Definition of chronic energy deficiency in adults. Report of a working party of the International Dietary Energy Consultancy Group. Eur J Clin Nutr. 1988;42:969-81.
21. James WPT, Schofield EC. Human energy requirements: a manual for planners and nutritionists. New York: Oxford University Press; 1990.
22. Leonard WR, Katzmarzyk PT, Stephen MA, Ross AG. Comparison of the heart rate-monitoring and factorial methods: assessment of energy expenditure in highland and coastal Ecuadoreans. Am J Clin Nutr. 1995;61:1146-52.
23. Morio B, Ritz P, Verdier E, Montaurier C, Beaufrere B, Vermorel M. Critical evaluation of the factorial and heart-rate recording methods for the determination of energy expenditure of free-living elderly people. Br J Nutr. 1997;78: 709-22.
24. Alfonzo-Gonzalez G, Doucet E, Almeras N, Bouchard C, Tremblay A. Estimation of daily energy needs with the FAO/WHO/UNU 1985 procedures in adults: comparison to whole-body indirect calorimetry measurements. Eur J Clin Nutr. 2004;58:1125-31.

Short Communication

Dietary energy requirements of young adult women in China by the doubly labeled water method

Jian-Min Liu PhD[1,2], Xiao-Guang Yang PhD[1], Jian-Hua Piao BSc[1], Rui Sun PhD[1], Ying Tian PhD[1]

[1]*Key Laboratory of Trace Element Nutrition MOH, Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention, Beijing, China*
[2]*Hebei Medical University, Shijiazhuang, China*

应用双标水法对中国青年女性膳食能量需要量的研究

目的：当前中国的能量参考摄入量(RNI)是一个估算值，它是以 WHO 推荐使用的 Schofield 公式计算基础代谢能量消耗，进而估算总能量需要量。由于缺乏可靠的针对中国人群的总能量消耗(TEE)及基础代谢能量消耗(BEE)的实测数据，因此其准确性值得商榷。本研究旨在对于中国青年女性的能量消耗值进行实际测定，并将实测值与推荐值加以比较分析。方法：以 16 名健康中国青年女性(年龄 22.1±1.2 岁，BMI 20.4±1.7)为研究对象，在严格控制实验条件的基础上，应用双标水法测定其总能量消耗，并应用气体代谢法测定基础代谢能量消耗。结果：测定的总能量消耗为 1380±118 kcal/d，与能量平衡观察法得到的膳食能量摄入值(1382±96 kcal/d)非常接近。将实测的总能量消耗值与相应目标人群的能量推荐值(2100 kcal/d)进行比较，结果发现推荐值比实测值高出 15%左右。同时，通过 Schofield 公式计算得到的基础代谢能量消耗预测值为 1230±65 kcal/d，比实测的基础代谢能量消耗值(1130±110 kcal/d)高出 9%左右。结论：中国青年女性能量参考摄入量应在实际测定目标人群总能量消耗及基础代谢能量消耗的基础上加以修正。

关键词：能量需要量、中国人、双标水、基础代谢能量消耗、总能量消耗