Supplementary Text S1. Study design and Participants

Eighteen healthy Chinese middle-aged adults (8 males, 10 females; aged 50－60 years) with a light physical activity profile were recruited. Inclusion criteria included normal body mass index (BMI 18.5~24 kg/m²) and stable body weight (fluctuations ≤ ± 2 kg) over the 3-month pre-enrollment period. Exclusion criteria comprised thyroid dysfunction, hepatic/renal impairment, metabolic disorders, individuals actively attempting weight loss or following a weight-loss diet within last 3 months prior to enrollment, and menstruating females. Participants avoided vigorous physical activity throughout the study.

Baseline assessments included comprehensive physical examinations with biochemical profiling. Fasting blood samples were analyzed for thyroid function (thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4)), liver enzymes (Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)), and renal function (Urea, Cr). Height (± 1 cm) and body weight (BW± 0.1 kg) were measured using calibrated equipment. Body composition was assessed by bioelectrical impedance analysis (BIA; MC-980MA, TANITA, Tokyo, Japan) to determine fat-free mass (FFM). BIA measurements were performed in the morning after an overnight fast (⩾8 hours) with an empty bladder. All participants wore light clothing, removed metal accessories, and maintained a standing posture in accordance with the manufacturer's standardized operating protocol (MC-980MA, TANITA, Tokyo, Japan). Measurements were conducted by a trained research assistant to ensure consistency.

To assess the participants' physical activity levels, they were required to wear an ActiGraph WGT3X-BT accelerometer (ActiGraph LLC, Fort Walton Beach, FL) at the waist for at least four consecutive days, with each participant achieving a minimum of 10 hours of valid wearing time per day. Water-based activities such as bathing were excluded during wearing periods. The accelerometer recorded physical activity at a sampling frequency of 60 Hz to obtain high-resolution movement data. The collected data were analyzed using ActiLife 6.0 software.

Supplementary Text S2. Doubly Labelled Water （DLW） Protocol for Energy Metabolism Measurement

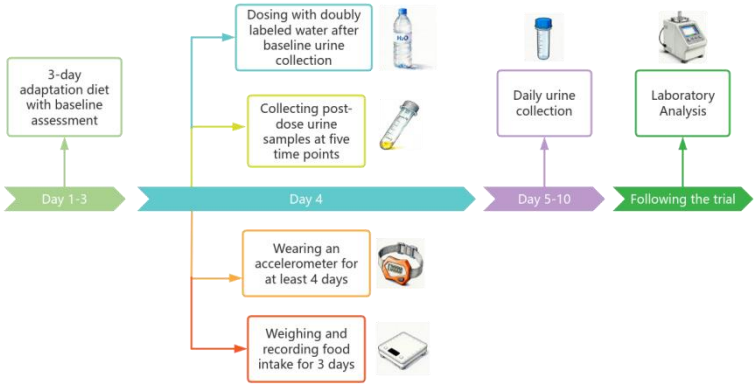
The DLW experimental protocol for the measurement of TEE was illustrated in Figure S1. The study was conducted over a 10-day period, comprising a 3-day adaptation phase followed by a 7-day testing phase. On the morning of day 4, after collecting a baseline urine sample, participants were administered an oral dose of 1 g $^{2}H_{2}^{18}O$ per kilogram of body weight. The dosage of doubly labelled water (DLW; ^{2}H: 10.3 atom%, ^{18}O: 8.3 atom%) was determined in accordance with guidelines established by the International Atomic Energy Agency (IAEA), and was further optimized based on the detection sensitivity and linear range of the isotope ratio mass spectrometry system employed in this study. The DLW was sourced from Jiangsu Changshu Huayi Chemical. Post-dose urine samples were collected at 1, 2, 3, 4, and 5 hours following administration and on the morning of the next 6 days. Tap water used for cooking was also collected as an isotopes baseline data of local area. Each urine and tap water sample was stored separately at -20°C until further analysis.

All urinary isotope analyses were performed at Createch Testing (Tianjin) using a Nu Perspective isotope ratio mass spectrometer (IRMS) with Gas Prep system and AS8000 autosampler. Hydrogen and oxygen isotopes were measured via H_2 - H_2O and CO_2 - H_2O equilibrium methods, respectively. International reference standards (VSMOW, SLAP) were used for calibration, and each sample was analyzed in six replicates. Data quality was ensured by adherence to the following criteria: $R^2 > 0.99$ for ^{2}H and ^{18}O elimination curves, a KO/KD ratio of 1.1–1.7, an ND/NO ratio of 1.00–1.07, and final-day isotope enrichment >50 ppm higher than baseline.

Supplementary Text S3. TEE calculation by the use of DLW (TEE-DLW)

TEE-DLW was calculated using a multi-point method in accordance with IAEA guidelines. The δ values of urine samples at each time point were converted to parts per million (ppm) for ^{18}O and ^{2}H. Baseline-corrected ppm excess values were derived

by subtracting pre-dose urinary ^{2}H and ^{18}O concentrations. The natural log (Ln) of the ^{18}O and ^{2}H excess values was plotted against time to generate elimination curves, from which the slopes (KD for ^{2}H and KO for ^{18}O) were obtained.



Supplementary Figure S1. Flowchart of the doubly labelled water (DLW) experimental protocol.

Extrapolating the curves to t = 0 yielded intercepts, which, following antilogarithm transformation and inversion, provided NO and ND, representing the metabolic pools of ^{2}H and ^{18}O, respectively. Using established formulas, the determination of total body water and the rate of CO_2 production (rCO_2) were calculated for each participant. In this study, the food quotient (FQ) was used as a substitute for the respiratory quotient (RQ) and incorporated into the TEE-DLW calculation: TEE-DLW (kcal/day) = rCO_2(L/day) × (1.106 + 3.94 / FQ)

In the above formula, the food quotient (FQ) was determined through the duplicate diet method. Participants were provided with a 3-day cyclic menu and were not permitted any other food or beverages. To quantify individual intake, the weight of each meal provided to and returned by every participant was accurately recorded. To implement the method, duplicate meals were collected over the full menu cycle, then homogenized, weighed, and stored at -20°C. Subsequent analysis determined the macronutrient composition (protein, fat, and carbohydrate) of these samples, which was used to calculate the FQ. The mean (± SD) FQ was 0.88 ± 0.01, determined via the duplicate diet method to obtain an objective, individual-specific

value, thereby enhancing TEE calculation accuracy by reflecting actual dietary composition.

Supplementary Table S1. Characteristics and TEE-DLW of the study participants

Characteristics	Total	Male	Female	*P*-value[a]
Sample size	18	8	10	-
Age (year)	55.6 ± 2.7	55.3 ± 2.5	55.8 ± 3.0	0.686
Height (cm)	161.4 ± 7.3	168.3 ± 5.1	155.9 ± 2.4	0.000
Weight (kg)	57.8 (56.1, 61.1)	61.3 (58.6, 69.8)	56.4 (54.1, 57.4)	0.000*
BMI (kg/m^2)	23.3 (21.7, 23.7)	22.1 (21.1, 23.6)	23.5 (22.5,23.7)	0.408*
FFM (kg)	38.2 (36.3, 47.9)	48.3 ± 4.0	36.4 ± 1.3	0.000
ALT (U/L)	15.5 (13.0, 20.8)	14.0 ± 2.9	20.2 ± 6.4	0.018
AST (U/L)	22.1 ± 4.1	18.0 (17.0, 21.0)	24.0 (21.8, 26.0)	0.016*
Urea (mmol/L)	5.5 ± 1.0	6.1 ± 0.5	5.0 ± 1.0	0.008
Cr (μmol/L)	60.8 ± 9.4	68.9 ± 4.0	54.4 ± 7.1	0.000
T3 (nmol/L)	1.8 ± 0.2	1.8 ± 0.3	1.7 ± 0.2	0.406
T4 (nmol/L)	104.8 (98.5, 107.9)	107.7 (98.7, 108.8)	103.6 (93.9, 105.6)	0.274*
TSH (mIU/L)	2.0 ± 0.9	1.6 ± 0.7	2.3 ± 0.9	0.073

Note. Values were expressed as mean ± SD or median (P_{25}, P_{75}) according to data distribution; [a]Comparison between male and female; *Mann-Whitney U test; BMI: body mass index; FFM: fat-free mass; ALT: Alanine aminotransferase, AST: Aspartate aminotransferase; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone.

Supplementary Table S2. Comparison of TEE-DLW between Chinese middle-aged and younger adults

Subjects	Sample size	Age (year)	Body Weight (kg)	Fat-free mass (kg)	TEE-DLW (kcal/d)
Male[Zhuo, 2013]	16	23.0 ± 1.0	64.9 ± 4.6	54.1 ± 3.5	2,258 ± 180^{ns}
Female[Liu, 2010]	16	22.1 ± 1.2	54.5 ± 4.7	41.3 ± 2.3	1,827 ± 118^{ns}
Current study					
Male	8	55.3 ± 2.5	61.3 (58.6, 69.8)	48.3 ± 4.0	2,267 ± 413
Female	10	55.8 ± 3.0	56.4 (54.1, 57.4)	36.4 ± 1.3	1,823 ± 181

Note. Values were expressed as mean ± SD or median (P_{25}, P_{75}), depending on distribution. Same-gender TEE comparisons across studies by using t-tests, ns: $P > 0.05$