Energy requirements of women of reproductive age¹⁻⁴

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ABSTRACT

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Background: The energy requirements of women have been based on total energy expenditure (TEE) derived from the factorial approach or as multiples of basal metabolic rate (BMR).

Objective: This study was designed to reevaluate the energy requirements of healthy, moderately active underweight, normal-weight, and overweight women of reproductive age.

Design: The energy requirements of 116 women [n = 13] with a low body mass index (BMI), n = 70 with a normal BMI, and n = 33with a high BMI] were estimated from TEE measured by the doubly labeled water method. Twenty-four-hour EE and BMR were measured by room respiration calorimetry, activity EE was estimated from nonbasal EE as TEE - BMR, and physical activity level was calculated as TEE/BMR. Body composition was derived from a multicomponent model. Fitness, strength, and physical activity level were assessed, and fasting serum indexes were measured. Results: Energy requirements differed among the low-BMI $(8.9 \pm 0.9 \text{ MJ/d})$, normal-BMI $(10.1 \pm 1.4 \text{ MJ/d})$, and high-BMI $(11.5 \pm 1.9 \text{ MJ/d})$ groups (P = 0.02–0.001, all pairwise comparisons). Major predictors of BMR, 24-h EE, and TEE were weight, height, and body composition; minor predictors were fasting metabolic profile and fitness. Fat-free mass and fat mass accounted for the differences in EE seen between the BMI groups. The mean physical activity level of 1.86 suggested that the multiples of BMR used to estimate energy requirements have been underestimated. Conclusion: Recommended energy intakes for healthy, moderately active women of reproductive age living in industrialized societies should be revised on the basis of TEE. Am J Clin Nutr 2003;77:630-8.

KEY WORDS Energy requirements, total energy expenditure, basal metabolic rate, activity, body composition, doubly labeled water method, women

INTRODUCTION

Traditionally, the energy requirements of adults have been based on total energy expenditure (TEE) derived from the factorial approach or as multiples of basal metabolic rate (BMR) (1, 2). The factorial approach requires information on the time spent in various activities and the energy cost of each activity. The factorial method is subject to errors of recorded or recalled time durations and of estimation of the energy cost and intensity of discrete and spontaneous activities. Energy requirements derived as multiples of BMR depend on the accuracy of the predicted or measured BMR and assignment into light, moderate, or heavy categories of physical activity. The doubly labeled water (DLW) method noninvasively measures TEE with the stable isotopes deuterium and oxygen-18 over several days (3). The DLW method has the advantage that it captures both BMR and the energy expended in physical activity and diet-induced thermogenesis. This approach for the assessment of TEE has been advocated and used by others (4–6). Black et al (4) compiled a large database of DLW measurements in persons aged 2–95 y from affluent societies. In their meta-analysis, TEE, BMR, and activity EE (AEE) were positively correlated with weight and height and negatively correlated with age. Females were found to have lower TEE than males. In a separate publication (5), TEE measurements by DLW were analyzed according to body mass index (BMI; in kg/m²), and physical activity level (PAL) was found to be similar across BMI groups.

The purpose of this study was to define the energy requirements of women of reproductive age based on TEE measured by the DLW method. Energy requirements are determined by biological as well as sociocultural factors and, therefore, are population-specific. Our subjects were representative of healthy, moderately active women of reproductive age living in an industrialized society. Our specific objectives were I) to determine the energy requirements of underweight, normal-weight, and overweight women; 2) to determine the effects of age, body composition, fasting metabolic profile, fitness, and strength on energy requirements; and 3) to determine significant predictors of BMR, 24-h EE, TEE, and AEE.

SUBJECTS AND METHODS

Subjects and study design

Subjects were classified as underweight, normal weight, or overweight: the low-BMI (≤ 18.5), normal-BMI (>18.5 but <25), and high-BMI (≥ 25) groups, respectively. Enrollment criteria included nonsmoking, ages 18–40 y, parity ≤ 4 , physically active (ie, 20–30 min moderate exercise ≥ 3 times/wk), and no chronic use of

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medications or alcohol or drug abuse. Fasting serum indexes, anthropometry, body composition, fitness, strength, respiration calorimetry, TEE, and physical activity were measured at the Children's Nutrition Research Center, Houston. This study was approved by the Baylor Affiliates Review Board for Human Subject Research, and written informed consent was obtained from each woman.

Serum indexes

A blood sample was obtained after the subjects had fasted for 12 h. Serum iron, iron saturation, and hemoglobin were determined by spectrophotometric methods. Hematocrit was measured by flow cytometry; ferritin by an automated chemiluminescence system (Bayer Corporation, Norwood, MA); transferrin receptor (TfR) by enzyme immunoassay (Ramco Laboratories, Inc, Houston); insulin and leptin by radioimmunoassay (Linco Research, Inc, St Charles, MO); glucose by an enzymatic method using glucose oxidase (EC 1.1.3.4); triacylglycerol by an enzymatic method using lipoprotein lipase (EC 3.1.1.34), glycerol kinase (EC 2.7.1.30), glycerol-1-phosphate dehydrogenase (EC 1.1.1.261), and diaphorase (EC 1.8.1.4); free fatty acids by an enzymatic method using acyl-CoA oxidase (EC 1.3.3.6); and thyrotropin, total and free thyroxine (T_3) , and total and free triiodothyronine (T₄) by radioimmunoassay (Diagnostic Products Corp, Los Angeles).

Anthropometry and body composition

Body weight and height were measured with an electronic balance (Healthometer, Bridgeview, IL) and stadiometer (Holtain Limited, Crymych, United Kingdom), respectively. Anthropometric measurements were made by a single investigator.

Total body water (TBW) was determined by dilution of an orally administered dose of deuterium oxide (100 mg ${}^{2}\text{H}_{2}\text{O/kg}$). Deuterium dilution space (N_{H}) was converted to TBW by dividing by 1.04. Body density was measured with an underwater weighing system that uses "force cube" transducers (Precision Biomedical Systems, Inc, State College, PA) (7). Body volume was corrected for residual lung volume measured by the simplified nitrogen washout method (8).

Dual-energy X-ray absorptiometry (DXA; QDR2000, software version 5.56; Hologic, Inc, Madison, WI) was used to measure total-body bone mineral content (BMC).

A 4-component body-composition model that uses body weight, TBW from ²H dilution, body volume from densitometry, and BMC from DXA was used to compute fat mass (FM) and fat-free mass (FFM) (9):

$$FM = 2.220 \text{ body volume} - 0.764 \text{ TBW} - 1.465 \text{ weight}$$
 (1)

$$FM = 2.747 \text{ body volume} - 0.71 \text{ TBW} + 1.46 \text{ BMC} - 2.05 \text{ weight}$$
 (2)

$$FFM = weight - FM$$
 (3)

where body volume and TBW are in L and FM, FFM, weight, and BMC are in kg.

Fitness and strength

Fitness was determined by measuring maximal oxygen consumption ($\dot{V}O_2$ max) on a cycle ergometer (Corival 400; Lode BV, Gronigen, Netherlands). The exercise protocol began with a constant power output (50 W) for 4 min, with the average of minutes 3–4 constituting the steady state. The power output was then increased every minute thereafter by 25 W. When the subject neared exhaustion, the power was increased by 15 W for 1 min. If the subject was able to continue further, power was increased by 10 W for 1 min. \dot{VO}_2 , heart rate, and respiratory quotient (RQ) were measured continuously. The Sensormedics 2900 metabolic cart (Yorba Linda, CA) used to collect the respiratory gases was calibrated before each test session.

Before the strength tests were conducted, the subjects were allowed to become familiar with the Cybex Multi-station and bench press equipment (Medway, MA). Instruction on each of the machines was followed by watching each woman complete the exercise with no resistance. Strength was assessed on the leg press, leg extension, bench press, and latissimus pull-down by the one-repetition-maximum test, defined as the maximum amount of weight that could be lifted successfully one time. Starting with a low-to-moderate weight, the subjects attempted lifts with gradually increased weights ($\approx 10\%$ at first, decreasing to 5% and 2.5% as difficulty became evident). Successive attempts were made with a 90-s rest period between attempts until failure occurred. Approximately 3–5 trials were needed to reach the one-repetition maximum.

Respiration calorimetry

Oxygen, carbon dioxide, and RQ were measured continuously in 31-m³ room calorimeters for 24 h. The performance of the respiration calorimeters was described in detail previously (10). Errors from 24-h infusions of nitrogen and carbon dioxide were $-0.34 \pm 1.24\%$ for \dot{VO}_2 and $0.11 \pm 0.98\%$ for \dot{VCO}_2 (10). Heart rate was recorded by telemetry (DS-3000; Fukuda Denshi, Tokyo), and physical activity was monitored by a Doppler microwave sensor (D9/50; Microwave Sensors, Ann Arbor, MI). The average temperature and humidity within the calorimeter were 23.4 ± 0.3 °C and $47.4 \pm 3.8\%$, respectively. All urine was collected during the 24-h calorimetry session. Urine samples were acidified with 6N HCl and refrigerated; urinary volume was measured and nitrogen concentrations determined by Kjeldahl digestion (Kjeltec Auto Analyzer 1030; Tecator, Hoganas, Sweden), followed by a phenol-hypochlorite colorimetric reaction (11). From the 24-h $\dot{V}O_2$, $\dot{V}CO_2$, and urinary nitrogen excretion, TEE was computed according to Livesey and Elia (12).

Subjects adhered to a set schedule while in the calorimeter. Calorimetry began at 0800. Meals were served at 0830, 1200, and 1730, with a snack at 1830. A morning and afternoon exercise session consisted of walking on a treadmill at 2.5 mph (\approx 4 km/h) at no grade for 15 min (905E; Precor, Bothell, WA). No food was allowed after 1900; bedtime was at 2200. After fasting overnight for 12 h, the subjects were awakened at 0645, were asked to void, and returned to sleep. The subjects were reawakened \approx 30 min later, and BMR was measured for 40 min. BMR was calculated by using the Weir equation (13).

Total energy expenditure measured with the doubly labeled water method

TEE used to define energy requirements was measured by the DLW method (3). After collection of a baseline saliva sample, the women received by mouth 100 mg ${}^{2}\text{H}_{2}\text{O}$ (Cambridge Isotope Laboratories, Andover, MA) and 125 mg H $_{2}{}^{18}\text{O}$ (Cambridge Isotope Laboratories) per kg body weight. One daily saliva sample was collected at home for the next 13 d and stored frozen at -20 °C in o-ring sealed vials. The time of collection was recorded.

Saliva samples were analyzed for hydrogen and oxygen isotope ratio measurements by gas isotope ratio mass spectrometry (14). For the hydrogen isotope ratio measurements, 10 μ L saliva without further treatment was reduced to hydrogen gas with 200 mg Zn reagent

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at 500 °C for 30 min (15). The ²H/¹H isotope ratios of the hydrogen gas were measured with a Finnigan Delta-E gas isotope ratio mass spectrometer (Finnigan MAT, San Jose, CA). For the oxygen isotope ratio measurements, 100 μ L saliva was allowed to equilibrate with 300 mbar CO₂ of known ¹⁸O content at 25 °C for 10 h with a VG ISOPREP-18 water-CO₂ equilibration system (VG Isogas, Limited, Cheshire, United Kingdom). At the end of the equilibration, the ¹⁸O/¹⁶O isotope ratios of the carbon dioxide were measured with a VG SIRA-12 gas isotope ratio mass spectrometer (VG Isogas).

The isotope dilution spaces for ²H ($N_{\rm H}$) and ¹⁸O ($N_{\rm O}$) were calculated as follows:

$$N_{\rm H} \text{ or } N_{\rm O} \text{ (mol)} = (d \times A \times E_a)/(a \times E_d \times 18.02)$$
 (4)

where *d* is the dose of ${}^{2}\text{H}_{2}\text{O}$ or $\text{H}_{2}{}^{18}\text{O}$ in g, *A* is the amount of laboratory water in g used in the dose dilution, *a* is the amount of ${}^{2}\text{H}_{2}\text{O}$ or $\text{H}_{2}{}^{18}\text{O}$ in g added to the laboratory water in the dose dilution, *E_a* is the increase in ${}^{2}\text{H}$ or ${}^{18}\text{O}$ abundance in the laboratory water after the addition of the isotopic water, and *E_d* is obtained from the zero-time intercepts of the ${}^{2}\text{H}$ and ${}^{18}\text{O}$ decay curves in the saliva samples.

Carbon dioxide production (VCO₂) was calculated from the fractional turnover rates of ²H ($k_{\rm H}$) and ¹⁸O ($k_{\rm O}$) as follows:

$$VCO_2 \text{ (mol/d)} = 0.4584 \times [(k_0 \times N_0) - (k_H \times N_H)]$$
 (5)

In this equation, the in vivo isotope fractional factors of 0.945 $[f_1, {}^{2}\text{H}_2\text{O}_{(\text{liquid})} \leftrightarrow {}^{2}\text{H}_2\text{O}_{(\text{gas})}]$, 0.990 $[f_2, \text{H}_2{}^{18}\text{O}_{(\text{liquid})} \leftrightarrow \text{H}_2{}^{18}\text{O}_{(\text{gas})}]$, and 1.039 $[f_3, \text{H}_2{}^{18}\text{O}_{(\text{liquid})} + \text{C}{}^{16}\text{O}_{2(\text{gas})} \leftrightarrow \text{H}_2{}^{16}\text{O}_{(\text{liquid})} + \text{C}{}^{18}\text{O}_{2(\text{gas})}]$ measured at 37 °C were used (16–19). $\dot{V}\text{CO}_2$ was converted to TEE by using the Weir equation (13) as follows:

TEE (MJ/d) =
$$22.4 \times (1.106 \times \dot{V}CO_2 + 3.941 \times \dot{V}O_2)/239$$
 (6)

where \dot{VO}_2 was calculated from the food quotient (FQ) of 0.86 by using the relation $\dot{VO}_2 = \dot{VCO}_2$ /FQ according to Black et al (20). The overall precision of the DLW method computed according to Cole and Coward (21) was 8.2%. AEE was estimated from nonbasal EE as TEE – BMR. The PAL was estimated as TEE/BMR.

Physical activity questionnaire

The Taylor Questionnaire for the Assessment of Leisure Time Physical Activities (22) was modified to include both leisure and occupational activities performed by women. The self-report questionnaire assessed the number of times per month (frequency) and time per occasion (duration) that the women spent in various activities categorized as walking, dancing, bicycling, conditioning exercise, water activities, winter activities, sports, lawn and garden activities, home activities, fishing and hunting, and occupational activities according to Ainsworth et al (23). The time in hours per month was multiplied by the intensity codes or metabolic equivalents (METs; defined as $3.5 \text{ mL O}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ or $4.184 \text{ kJ} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) for specific activities published by Ainsworth et al (23). METs were summed across all activities and expressed as total METs (per kg/mo). Each woman's occupation was also assigned an MET value for classification purposes (23).

Statistics

MINITAB (release 13, 1998; Minitab Inc, College Station, PA) was used for data description and statistical analyses, including Pearson's correlation coefficients, paired t test, chi-square test, and linear regression. Analysis of variance was used to test for differences in outcome variables between the BMI groups; the model

included subjects and covariates of weight, FFM, and FM in some applications. Post hoc pairwise comparisons between groups were performed by using Tukey's method.

RESULTS

Subject description

Of the total of 116 women studied, 13 were underweight, 70 were of normal weight, and 33 were overweight. No significant differences in age, ethnicity, or family income were observed among the low-, normal-, and high-BMI groups. The mean age of the subjects was 31 ± 4 y (range: 21–40 y). The ethnic distribution was 78% white, 11% African American, 9% Hispanic, and 2% Asian. Family income was >\$50 000 in 80% of the sample. Mean education attainment was 17 ± 2 y, and was slightly lower in the high-BMI group than in the other groups ($P \le 0.04$). Sixty-seven percent of the women were nulliparous, 28% had one child, 4% had 2 children, and 1% had 3 children. Most (91%) of the women worked outside of the home: 36% were in business or administrative positions in an office setting; 6% worked in laboratories; 22% were teachers, professors, or students; 20% were health care providers; 7% were physical trainers; and 9% were homemakers.

The women were nonanemic, normoglycemic, and euthyroidic. Hemoglobin, hematocrit, serum iron, iron saturation, ferritin, TfR, and TfR/ferritin were within normal limits for women of reproductive age (Table 1). Serum iron (P = 0.02) and iron saturation (P = 0.05) were significantly lower in the low-BMI group than in the other 2 groups. TfR was higher in the high-BMI group than in the normal-BMI group (P = 0.04). Although the women were normoglycemic, serum insulin (P = 0.001) and glucose (P = 0.008) were significantly higher in the high-BMI group than in the other 2 groups. Serum leptin was higher in the high-BMI group than in the other 2 groups (P = 0.001). Serum triacylglycerol was higher in the high-BMI group than in the normal-BMI group (P = 0.001). BMI, weight, FM, and %FM were significantly correlated with insulin (r = 0.68 - 0.74, P = 0.001), leptin (r = 0.80 - 0.87, P = 0.001), leptin (rP = 0.001), triacylglycerol (r = 0.32-0.43, P = 0.001), glucose (r = 0.31 - 0.36, P = 0.001), and thyrotropin (r = 0.19 - 0.22, P = 0.05).

Anthropometry and body composition

Body size, dimension, and composition are presented by BMI group in **Table 2**. Except for height, highly significant differences for all variables were seen among the low-, normal- and high-BMI groups.

Fitness and strength

Approximately 41% of the variance in \dot{VO}_2 max could be explained by FFM and FM, with FM having a significant negative effect (**Table 3**). Absolute \dot{VO}_2 max tended to differ by BMI group (P = 0.08), whereas the maximal workload achieved was significantly different (P = 0.02); the low-BMI group tended to perform more poorly in terms of \dot{VO}_2 max than did the other groups (P = 0.07-0.100). The differences among BMI groups were accounted for by weight or FFM and FM. \dot{VO}_2 max was predicted by using Equation 7 [SEE = 0.30, r^2 (adjusted) = 40.8%]:

$$\dot{V}O_2$$
max (L/min) = 0.10 + 0.05 FFM (kg)
- 0.02 FM (kg) (7)

Testing showed differences in strength among the BMI groups. The weights lifted in the leg extension (P = 0.02) and bench press (P = 0.008) were greater in the high- than in the low-BMI group.

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TABLE I				
Fasting serum	indexes	by	BMI	grouping

	Low BMI $(n = 13)$	Normal BMI $(n = 70)$	High BMI $(n = 33)$	P (ANOVA)
Iron (µmol/L)	14.7 ± 3.1^{a}	19.8 ± 5.5 ^b	$19.7 \pm 5.3^{\rm b}$	0.008
Iron saturation (%)	$26.2 \pm 5.6^{\mathrm{a}}$	34.6 ± 9.8^{b}	33.7 ± 9.5^{b}	0.02
Hemoglobin (g/L)	136 ± 10	137 ± 8	139 ± 8	0.38
Hematocrit (%)	40.1 ± 3.1	40.4 ± 2.5	41.1 ± 2.5	0.30
Ferritin (µg/L)	37.0 ± 21.9	50.2 ± 40.2	47.1 ± 28.5	0.53
TfR (mg/L)	$4.7 \pm 1.1^{a,b}$	4.6 ± 1.3^{a}	$5.4 \pm 1.8^{\rm b}$	0.05
TfR/ferritin (μg/μg)	177 ± 125	161 ± 138	181 ± 178	0.82
Insulin (pmol/L)	37.2 ± 11.1^{a}	51.5 ± 21.2^{a}	101.1 ± 40.9^{b}	0.001
Glucose (mmol/L)	4.6 ± 0.3^{a}	4.7 ± 0.5^{a}	$5.0 \pm 0.4^{\rm b}$	0.002
Insulin/glucose (pmol/mmol)	8.0 ± 2.2^{a}	10.9 ± 4.6^{a}	$19.9 \pm 7.2^{\rm b}$	0.001
Leptin (ng/mL)	6.4 ± 3.6^{a}	9.6 ± 6.3	30.4 ± 14.9^{b}	0.001
Triacylglycerol (mmol/L)	$1.18 \pm 0.43^{a,b}$	1.07 ± 0.33^{a}	1.52 ± 0.73^{b}	0.001
Free fatty acids (mmol/L)	0.52 ± 0.18	0.55 ± 0.19	0.59 ± 0.21	0.42
Thyrotropin (mU/L)	1.07 ± 0.40	1.35 ± 0.64	1.58 ± 0.75	0.10
Total T_4 (nmol/L)	95 ± 19	94 ± 22	96 ± 19	0.93
Free T_4 (nmol/L)	0.02 ± 0.004	0.02 ± 0.003	0.02 ± 0.002	0.87
Total T_3 (nmol/L)	1.72 ± 0.31	1.74 ± 0.24	1.70 ± 0.24	0.73
Free T ₃ (nmol/L)	0.005 ± 0.001	0.005 ± 0.001	0.005 ± 0.001	0.69

 ${}^{I}\bar{x} \pm$ SD. TfR, transferrin receptor, T₄, thyroxine; T₃, triiodothyronine. Means within a row with different superscript letters are significantly different, P < 0.05 (pairwise comparisons by Tukey's simultaneous tests).

Differences in strength were accounted for by differences in FFM and FM among the BMI groups.

Basal metabolic rate

BMR did not differ significantly by age (range: 21–40 y) (**Table 4**). BMR (kJ/d) was significantly correlated (P = 0.001) with weight (r = 0.78), height (r = 0.43), BMI (r = 0.68) (**Figure 1**), FFM (r = 0.72), FM (r = 0.65), and %FM (r = 0.49). In linear regression models, BMR was significantly predicted by weight and height [Equation 8; SEE = 422, r^2 (adjusted) = 63.8%] or FFM and FM [Equation 9; SEE = 420, r^2 (adjusted) = 63.9%]. Addition of quadratic terms to the models did not improve the predictions.

BMR
$$(kJ/d) = -950 + 36.8$$
 weight (kg)
+ 2561 height (m) (8)

BMR
$$(kJ/d) = 2168 + 66.4$$
 FFM $(kg) + 28.4$ FM (kg) (9)

BMR was predicted from weight and height by using Schofield equations (24) for women aged 18–30 y or 30–60 y. Predicted BMR was significantly higher than the measured value for the younger women (predicted – measured = 402 ± 381 kJ/d; $7.8 \pm 7.3\%$ of measured; P = 0.001) but not for the older women $(-25 \pm 431$ kJ/d; $0.2 \pm 9.1\%$; NS).

Fasting serum glucose (r = 0.33, P = 0.001), triacylglycerol (r = 0.36, P = 0.001), free fatty acids (r = 0.23, P = 0.01), insulin (r = 0.53,

P = 0.001), thyrotropin (r = 0.28, P = 0.005), TfR (r = 0.36, P = 0.001), and leptin (r = 0.50, P = 0.001) were significantly correlated with BMR. After adjustment for FFM and FM, only free fatty acids (P = 0.007) were significantly correlated with BMR; correlations of BMR with leptin (P = 0.06) and thyrotropin (P = 0.07) were nearly significant. BMR was not significantly correlated with total or free T₃ and T₄. Absolute $\dot{V}O_2$ max was significantly correlated with BMR (r = 0.37, P = 0.001). An effect of $\dot{V}O_2$ max, adjusted for weight and height or FFM and FM, on BMR was shown. In a stepwise regression, weight, $\dot{V}O_2$ max, and fasting serum thyrotropin, free fatty acids, and leptin were entered as significant predictors of BMR [r^2 (adjusted) = 68.1%].

Twenty-four-hour energy expenditure measured by room respiration calorimetry

Twenty-four-hour EE (kJ/d) was significantly correlated (P = 0.001) with weight (r = 0.80) (**Figure 2**), height (r = 0.35), BMI (r = 0.73), FFM (r = 0.73), FM (r = 0.69), %FM (r = 0.53), and BMR (r = 0.89). In linear regression models, 24-h EE was significantly predicted by weight and height [Equation 10; SEE = 586, r^2 (adjusted) = 64.2%] or FFM and FM [Equation 11; SEE = 569, r^2 (adjusted) = 66.2%]. Addition of quadratic terms to the models did not improve the predictions.

24-h EE (kJ/d) =
$$1238 + 55.6$$
 weight (kg)
+ 16.7 height (m) (10)

TABLE 2				
Anthropometric and b	ody-composition	measures b	y BMI	grouping ¹

1 5 1				
	Low BMI (<i>n</i> = 13)	Normal BMI $(n = 70)$	High BMI $(n = 33)$	P (ANOVA)
Weight (kg)	49.2 ± 3.6^{a}	57.5 ± 6.0^{b}	$80.8 \pm 10.5^{\circ}$	0.001
Height (m)	1.66 ± 0.06^{a}	1.64 ± 0.05^{b}	$1.65 \pm 0.06^{\circ}$	0.71
BMI (kg/m^2)	17.9 ± 0.5^{a}	$21.2 \pm 1.7^{\rm b}$	$29.6 \pm 3.4^{\circ}$	
Body composition				
Fat-free mass (kg)	38.6 ± 3.6^{a}	42.1 ± 4.4^{b}	$48.0 \pm 5.6^{\circ}$	0.001
Fat mass (kg)	10.6 ± 2.5^{a}	15.4 ± 4.3^{b}	$32.8 \pm 8.2^{\circ}$	0.001
Fat mass (% of body wt)	21.6 ± 4.8^{a}	26.6 ± 5.9^{b}	$40.2 \pm 6.4^{\circ}$	0.01

 $^{1}\overline{x} \pm$ SD. Means within a row with different superscript letters are significantly different, P < 0.05 (pairwise comparisons by Tukey's simultaneous tests).

TABLE 3

Physical fitness and strength by BMI grouping¹

	Low BMI $(n = 13)$	Normal BMI $(n = 70)$	High BMI $(n = 33)$	P (ANOVA)
Submaximal exercise on treadmill at ≈4 km/h				
$\dot{V}O_2$ (L/min)	0.60 ± 0.06^{a}	0.69 ± 0.10^{a}	$0.92 \pm 0.14^{\rm b}$	0.001
Heart rate (bpm)	$94 \pm 15^{a,b}$	97 ± 13^{a}	110 ± 12^{b}	0.001
RQ	0.92 ± 0.03	0.91 ± 0.03	0.92 ± 0.03	0.18
Submaximal exercise on stationary cycle ergometer at 50 W				
\dot{VO}_2 (L/min)	$0.86\pm0.08^{\mathrm{a}}$	0.91 ± 0.08^{b}	$1.04 \pm 0.09^{\circ}$	0.001
Heart rate (bpm)	126 ± 17	120 ± 17	127 ± 16	0.17
RQ	0.96 ± 0.08	0.94 ± 0.07	0.96 ± 0.06	0.18
Maximal exercise on stationary cycle ergometer				
Workload (W)	138 ± 36^{a}	164 ± 35^{b}	$150\pm32^{a,b}$	0.02
[.] VO ₂ max (L/min)	1.76 ± 0.41	1.99 ± 0.39	2.04 ± 0.34	0.08
Heart rate (bpm)	178 ± 11	178 ± 8	180 ± 12	0.61
RQ	1.23 ± 0.06	1.22 ± 0.07	1.20 ± 0.08	0.42
Strength testing: one-repetition maximum				
Leg press (kg)	$28.2 \pm 13.2^{a,b}$	36.8 ± 17.3^{a}	28.6 ± 14.5^{b}	0.02
Leg extension (kg)	35.0 ± 7.3^{a}	$39.5 \pm 9.1^{a,b}$	43.2 ± 9.1^{b}	0.02
Bench press (kg)	29.1 ± 8.6^{a}	$34.1 \pm 7.2^{a,b}$	$37.3 \pm 7.7^{\rm b}$	0.009
Latissimus pull-down (kg)	27.3 ± 5.9	28.6 ± 5.0	30.9 ± 5.0	0.07

 ${}^{I}\bar{x} \pm$ SD. $\dot{V}O_2$, oxygen consumption; $\dot{V}O_2$ max, maximal oxygen consumption; bpm, beats per minute; RQ, respiratory quotient. Means within a row with different superscript letters are significantly different, P < 0.05 (pairwise comparisons by Tukey's simultaneous tests).

24-h EE (kJ/d) =
$$2799 + 89.5$$
 FFM (kg)
+ 42.7 FM (kg) (11)

or T₄. Absolute $\dot{V}O_2$ max (r = 0.38, P = 0.001) correlated significantly with 24-h EE. An effect of $\dot{V}O_2$ max, adjusted for weight and height or FFM and FM, on 24-h EE was shown. In a stepwise regression, weight, $\dot{V}O_2$ max, and fasting serum thyrotropin and latissimus pulldown were significant predictors of 24-h EE [r^2 (adjusted) = 67.3%].

Fasting serum glucose (r = 0.29, P = 0.002), triacylglycerol (r = 0.37, P = 0.001), free fatty acids (r = 0.19, P = 0.04), insulin (r = 0.48, P = 0.001), thyrotropin (r = 0.30, P = 0.002), serum iron (r = 0.22, P = 0.02), TfR (r = 0.28, P = 0.004), and leptin (r = 0.54, P = 0.001) were significantly correlated with 24-h EE. After adjustment for FFM and FM, only free fatty acids (P = 0.005) and thyrotropin (P = 0.03) were correlated with 24-h EE. Twenty-four-hour EE was not significantly correlated with total and free T₃

Energy cost of walking

The energy cost of walking at 4 km/h on a treadmill in the calorimeter was 12.6 ± 1.2 , 14.2 ± 2.2 , and 19.1 ± 2.9 kJ/min for the low-, normal- and high-BMI groups, respectively (**Figure 3**). The absolute energy cost of walking at 4 km/h differed between

TABLE 4

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Energy expenditure by (BMI) grouping¹

	Low BMI $(n = 13)$	Normal BMI $(n = 70)$	High BMI $(n = 33)$	P (ANOVA)
Calorimetry				
BMR (kJ/d)	4874 ± 553^{a}	5431 ± 484^{b}	$6291 \pm 618^{\circ}$	0.001
RQ	0.80 ± 0.07	0.81 ± 0.05	0.81 ± 0.05	0.84
24-h EE (kJ/d)	6525 ± 669^{a}	7235 ± 612^{b}	$8592 \pm 851^{\circ}$	0.001
RQ	0.88 ± 0.03	0.87 ± 0.02	0.87 ± 0.02	0.42
24-h EE/BMR	1.34 ± 0.07	1.34 ± 0.08	1.37 ± 0.09	0.66
Doubly labeled water				
$N_{\rm H}$ (kg)	29.4 ± 3.4^{a}	$32.5 \pm 3.7^{\rm b}$	$36.8 \pm 4.8^{\circ}$	0.001
$N_{\rm O}$ (kg)	28.5 ± 3.5^{a}	31.3 ± 3.5^{b}	$35.8 \pm 4.7^{\circ}$	0.001
$N_{\rm H}/N_{\rm O}$	1.03 ± 0.02	1.04 ± 0.02	1.03 ± 0.02	0.06
$k_{\rm H} ({\rm d}^{-1})$	-0.102 ± 0.03	-0.104 ± 0.04	-0.102 ± 0.04	0.96
$k_{\rm O} ({\rm d}^{-1})$	-0.128 ± 0.03	-0.132 ± 0.04	-0.129 ± 0.04	0.91
$k_{\rm H}/k_{\rm O}$	0.78 ± 0.06	0.78 ± 0.04	0.78 ± 0.05	0.90
$\dot{V}CO_2$ (L/d)	372 ± 39^{a}	425 ± 60^{b}	$484 \pm 79^{\circ}$	0.001
TEE (kJ/d)	8864 ± 923^{a}	$10111 \pm 1421^{\rm b}$	$11525 \pm 1884^{\circ}$	0.001
Physical activity				
AEE (kJ/d)	3990 ± 934^{a}	$4691 \pm 1365^{a,b}$	5313 ± 1634^{b}	0.02
PAL	1.84 ± 0.27	1.87 ± 0.26	1.86 ± 0.26	0.89

 ${}^{I}\bar{x} \pm$ SD. RQ, respiratory quotient; $N_{\rm H}$ and $N_{\rm O}$, isotope dilution spaces; $k_{\rm H}$ and $k_{\rm O}$, fractional turnover rates of 2 H and 18 O; \dot{V} CO₂, carbon dioxide production; BMR, basal metabolic rate; EE, energy expenditure; TEE, total energy expenditure; AEE, activity energy expenditure; PAL, physical activity level (PAL = TEE/BMR). Means within a row with different superscript letters are significantly different, P < 0.05 (pairwise comparisons by Tukey's simultaneous tests).



FIGURE 1. Basal metabolic rate as a function of weight in women classified into low-BMI (n = 13; \bullet), normal-BMI (n = 70; \bigcirc), or high-BMI (n = 33; +) categories (r = 0.78, P = 0.001).

BMI groups; however, the differences were not significant after adjustment for weight or FFM and FM. The energy cost of walking at 4 km/h is described as a function of body weight (height was not significant) in Equation 12 [SEE = 1.92, r^2 (adjusted) = 65.4%] and as a function of FFM and FM in Equation 13 [SEE = 1.95, r^2 (adjusted) = 65.1%].

Energy cost of walking (kJ/min)
=
$$2.84 + 0.201$$
 weight (kg) (12)

Energy cost of walking
$$(kJ/min) = 3.24$$

+ 0.188 FFM $(kg) + 0.204$ FM (kg) (13)

Free-living total energy expenditure: basis of energy requirements

TEE and, therefore, energy requirements were not affected significantly by age. TEE was significantly correlated (P = 0.01-0.001) with weight (r = 0.54) (**Figure 4**), height (r = 0.26), BMI (r = 0.48), FFM (r = 0.58), FM (r = 0.41), %FM (r = 0.24), and BMR (r = 0.56). TEE was predicted by Equations 14 [SEE = 1435, r^2 (adjusted) = 29.3%] and 15 [SEE = 1381, r^2 (adjusted) = 34.5%]

$$TEE (kJ/d) = 874 + 67.4 \text{ weight (kg)} + 3192 \text{ height (m)}$$
(14)



FIGURE 2. Twenty-four-hour energy expenditure as a function of weight in women classified into low-BMI ($n = 13; \bullet$), normal-BMI ($n = 70; \bigcirc$), or high-BMI ($n = 33; \bullet$) categories (r = 0.80, P = 0.001).



FIGURE 3. Energy cost of walking as a function of weight in women classified into low-BMI (n = 13; \bullet), normal-BMI (n = 70; \bigcirc), or high-BMI (n = 33; \bullet) categories (r = 0.81, P = 0.001).

TEE (kJ/d) = 3251 + 149 FFM (kg) + 32.6 FM (kg) (15)

TEE was significantly correlated with fasting serum triacylglycerol (r = 0.24, P = 0.01), free fatty acids (r = 0.20, P = 0.04), insulin (r = 0.28, P = 0.004), thyrotropin (r = 0.29, P = 0.005), serum iron (r = 0.22, P = 0.03), and leptin (r = 0.35, P = 0.001). After adjustment for FFM and FM, only free fatty acids (P = 0.03) and thyrotropin (P = 0.02) were correlated with TEE. TEE was not significantly correlated with total T₃ or total and free T₄. Absolute \dot{VO}_2 max (r = 0.42, P = 0.001) significantly correlated with TEE. An independent effect of \dot{VO}_2 max on TEE was shown, independent of body size and composition. In a stepwise regression, FFM, fasting serum thyrotropin, \dot{VO}_2 max, and FM were significant predictors of TEE [r^2 (adjusted) = 48.5\%].

AEE was significantly correlated with weight (r = 0.29, P = 0.002), BMI (r = 0.26, P = 0.007), FFM (r = 0.34, P = 0.001), FM (r = 0.20, P = 0.04), \dot{VO}_2 max (r = 0.30, P = 0.002), leptin (r = 0.20, P = 0.05), thyrotropin (r = 0.2, P = 0.04), and self-reported total METs (per kg/mo) (r = 0.27, P = 0.006). The ability to predict AEE from weight and height [r^2 (adjusted) = 7.0%] or FFM and FM [r^2 (adjusted) = 10.3%] was limited. In a stepwise regression,



FIGURE 4. Total energy expenditure as a function of weight in women classified into low-BMI (n = 13; \bullet), normal-BMI (n = 70; \bigcirc), or high-BMI (n = 33; +) categories (r = 0.54, P = 0.001).

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TABLE 5

Characteristics of the women by physical activity level (PAL) quartile $(Q)^{1}$

	Q1, PAL < 1.70	Q2, $170 \le PAL < 1.85$	Q3, $1.85 \le PAL < 2.00$	Q4, PAL ≥ 2.00	Р
	(n = 29)	(n = 29)	(n = 29)	(n = 29)	(ANOVA)
PAL	1.56 ± 0.14	1.78 ± 0.04	1.92 ± 0.04	2.20 ± 0.18	_
Age (y)	30.8 ± 3.3	30.6 ± 4.4	31.2 ± 3.9	31.0 ± 4.9	0.95
Weight (kg)	59.4 ± 8.4	63.7 ± 12.6	67.0 ± 15.5	60.1 ± 13.6	0.11
Height (m)	1.65 ± 5.2	1.65 ± 5.6	1.64 ± 6.0	1.64 ± 5.8	0.90
BMI (kg/m ²)	21.9 ± 3.3	23.3 ± 4.1	24.8 ± 5.6	22.1 ± 4.5	0.06
FFM (kg)	41.3 ± 4.3	44.4 ± 5.7	44.7 ± 6.2	43.2 ± 5.9	0.11
FM (kg)	18.1 ± 6.7	19.3 ± 8.7	22.3 ± 12.0	16.9 ± 9.0	0.18
%FM (% of body wt)	29.8 ± 7.2	29.2 ± 7.8	31.4 ± 10.5	26.8 ± 7.7	0.25
24 h EE (kJ/d)	7314 ± 962	7744 ± 887	7778 ± 1109	7230 ± 820	0.08
BMR (kJ/d)	$5565\pm573^{a,b}$	5778 ± 628^{a}	$5694 \pm 715^{a,b}$	5234 ± 632^{b}	0.02
TEE (kJ/d)	8661 ± 962^{a}	10267 ± 887^{b}	$10920 \pm 1109^{b,c}$	$11514 \pm 820^{\circ}$	0.001
AEE (kJ/d)	2230 ± 686^a	3489 ± 368^{b}	$4134 \pm 515^{\circ}$	5130 ± 1163^{d}	0.001
^V O₂max (L/min)	1.81 ± 0.28^{a}	2.09 ± 0.33^{b}	$2.04 \pm 0.42^{a,b}$	$2.03 \pm 0.46^{a,b}$	0.04
Leg press (kg)	30.0 ± 13.6	39.5 ± 18.2	34.1 ± 16.4	34.5 ± 16.4	0.3
Leg extension (kg)	39.5 ± 10.0	41.4 ± 8.6	40.5 ± 8.6	39.1 ± 10.0	0.8
Bench press (kg)	$31.4 \pm 8.2^{\mathrm{a}}$	37.3 ± 8.2^{b}	$36.4 \pm 8.2^{\mathrm{a,b}}$	$33.2\pm7.3^{\mathrm{a,b}}$	0.03
Latissimus pull-down (kg)	27.7 ± 5.0	31.4 ± 4.5	29.1 ± 5.9	29.5 ± 4.5	0.11
Total METs (per kg/mo)	614 ± 287	630 ± 252	785 ± 305	799 ± 296	0.04

 ${}^{I}\bar{x} \pm$ SD. FFM, fat-free mass; FM, fat mass; EE, energy expenditure; BMR, basal metabolic rate; TEE, total energy expenditure; AEE, activity energy expenditure; $\dot{V}O_2$ max, maximal oxygen consumption; MET, metabolic equivalents. Means within a row with different superscript letters are significantly different, P < 0.05 (pairwise comparisons by Tukey's simultaneous tests).

FFM and total METs (per kg/mo) were significant predictors of AEE [r^2 (adjusted) = 17.6%].

Energy expenditure and energy requirements by body mass index

The EE of the women categorized by BMI is presented in Table 4. BMR, 24-h EE, AEE (P = 0.05), TEE (P = 0.001), and, therefore, energy requirements, differed among BMI groups. After adjustment for weight and height, BMR and 24-h EE were lower in the low-BMI group than in the normal-BMI and high-BMI groups (P < 0.04). After adjustment for FFM and FM, BMR and 24-h EE did not differ significantly between the BMI groups. After adjustment for weight and height or FFM and FM, TEE and AEE did not differ significantly between the BMI groups. PAL did not differ significantly between the BMI groups.

Physical activity level

The mean PAL of the women was 1.86 ± 0.26 (quartile 1: 1.70; quartile 2: 1.85; quartile 3: 2.00). PAL did not differ significantly by age. A description of the women by PAL category is presented in **Table 5**. Age, body size, and body composition were not significantly different between the women in the 4 PAL quartiles. As expected, differences in BMR, TEE, AEE, and \dot{VO}_2 max were detected between the PAL quartiles (P = 0.04-0.001). Fasting serum indexes did not differ significantly by PAL quartile.

The occupations of the women were assigned a categorical MET value that varied from 1.5 to 4.5 (23). Occupations were classified as sedentary (MET = 1.5 for business and administrative positions in an office setting), moderately active (MET = 2.0-2.5 for laboratory technician, teachers, professors, and students), active (MET = 3.0 for homemakers and health care providers), and very active (MET = 4.5 for physical trainers). No significant differences in TEE, AEE, or PAL were evident between the women by occupation.

According to the Taylor Questionnaire for the Assessment of Leisure Time Physical Activities, the women spent a total of 720 ± 299 METs (per kg/mo) in the following physical activities: occupational activities (278 \pm 219), home activities (221 \pm 205), conditioning exercise (97 \pm 108), walking (60 \pm 136), dancing (26 ± 61) , water activities (13 ± 31) , lawn and garden activities (13 ± 1) 21), sports (12 ± 36), bicycling (10 ± 36), winter activities (5 ± 39), and fishing and hunting (0.4 ± 3.2) . Total METs (per kg/mo) were positively correlated with PAL (r = 0.28, P = 0.005). The energy equivalent of the reported METs for the various activities $(6297 \pm 2933 \text{ kJ/d})$ was positively correlated with TEE (r = 0.46, P = 0.001) and AEE (r = 0.38, P = 0.001) estimated from the DLW method. Total METs (per kg/mo) did not differ significantly by BMI group. With regard to specific activities, women in the normal-BMI group spent more time doing conditioning exercises than did women in the other 2 groups (P = 0.02). Total METs (per kg/mo) differed between the women in the 4 PAL quartiles (P = 0.04). Women in the highest PAL quartile participated in bicycling to a greater extent (P = 0.006) than did the women in the other groups; they also tended (P < 0.10) to spend more time in conditioning and water activities than did the women in the other groups.

DISCUSSION

The purpose of this study was to determine the energy requirements of healthy underweight, normal-weight, and overweight women of reproductive age on the basis of TEE. The effects of age, body composition, fasting metabolic profile, fitness, strength, and occupational and recreational activities on TEE and its components were examined. Major predictors of BMR, 24-h EE, and TEE were body size and body composition; minor predictors were fasting metabolic profile and fitness. FFM and FM accounted for the differences in BMR, 24-h EE, and TEE seen between the underweight, normalweight, and overweight women. The predictability of AEE and PAL of these women was low; no significant differences in age, body size, body composition, or serum indexes were detected by PAL quartile.

The approach taken to define the energy requirements of women was based on TEE measured by the DLW method. The definition of energy requirements is population-specific. For this reason, we carefully described the health status, anthropometric indexes, body composition, fitness, and lifestyles of our subjects. Our study population consisted of healthy, moderately active women living in an urban, industrialized setting; most of the women worked outside of the home, 33% had young children, and most participated in moderate exercise ≥ 3 times/wk. By design, the women represented a wide spectrum of body sizes and compositions. In terms of strength and \dot{VO}_2 max, the women in the low-BMI group did not perform as well as did the other groups.

Factors influencing the energy expenditure of women

To understand the variability in energy requirements of the women, we and others explored the effects of age, body size, and body composition on EE (4–6, 25–30). EE declines with age throughout life (4, 30), but we did not see a significant decline within our subjects' limited age span of 21–40 y. The effects of body size and composition on BMR, 24-h EE, TEE, and AEE were examined in our study. Body size or composition accounted for 64% of the variance in BMR, 64–66% of the variance in 24-h EE, 29–34% of the variance in TEE, and 7–10% of the variance in AEE. The lower predictability of TEE and AEE was due to the fact that activity patterns are influenced by behavioral choices and, therefore, are less definable with the use of biological measures. To better predict TEE and AEE, we tested physical activity–related variables, such as leisure time activities, \dot{VO}_2 max and strength, which slightly improved the prediction of TEE and AEE.

We found minor contributions of fasting serum hormones and metabolites to the variance observed in EE. Independent of FFM and FM, free fatty acids and thyrotropin were related to BMR, 24-h EE, and TEE. The positive association between fasting serum free fatty acids and rates of EE may reflect higher free fatty acid flux, oxidation, or both. Thyrotropin is a stimulator of T_3 and T_4 release, which in turn increase \dot{VO}_2 and heat production.

Energy expenditure by body mass index

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Absolute rates of BMR, 24-h EE, TEE, and AEE were substantially higher in the women who were overweight than in those who were not, as was found by other investigators (5, 26, 27, 31). After adjustment for body size or composition, no significant differences in EE were found between BMI groups. PAL has been shown to be similar between BMI categories in women and men (5, 27). The higher 24-h EE and TEE values observed in the overweight women were attributable to the higher BMR and energy cost of physical activities, as exemplified by the cost of cycling at 50 W and walking at 4 km/h in the calorimeter, which were 14% and 21% higher, respectively, in the high-BMI group than in the normal-BMI group. Although PALs were similar among BMI groups, the amount of time spent in comparable physical activities would be less in the overweight women. For instance, if the observed AEE entailed only walking at 4 km/h, the duration of walking would be equivalent to 324, 332, and 285 min in the low-, normal- and high-BMI groups, respectively.

Energy requirements based on total energy expenditure and physical activity level

PAL provides a convenient way of controlling for age, sex, weight, and body composition. To validate the PAL index, regression of the logarithms of 24-h EE and TEE on the logarithms of BMR yields coefficients of 0.91 and 0.73, indicating that the ratio approach in this case does completely adjust for BMR. In an analysis by Black et al (4), the logarithm of TEE was regressed on the logarithm of BMR in 574 adults from affluent societies. The resultant regression coefficients were 1.00 for all subjects, 0.98 for males, and 0.99 for females. These findings indicated that the PAL index was not correlated with BMR and was thus a valid index of TEE adjusted for BMR. The larger sample size in the study by Black et al favors its findings. The PAL provides a useful index of physical activity and a practical approach for estimating energy requirements.

Our results suggest that the multiples of BMR used to estimate the energy requirements of moderately active women have been underestimated in the 1985 FAO/WHO/UNU energy and protein requirements (1) and in the 1989 US recommended dietary allowances (2). In the FAO/WHO/UNU publication, multiples of 1.56, 1.64, and 1.82 were used to represent light, moderate, and heavy PALs in women. In the US recommended dietary allowances, activity factors of 1.60 and 1.55 were assigned to women aged 19-24 and 25-50 y, respectively, engaged in light-to-moderate activity. In our study, the mean PAL within the calorimeter was 1.35, representing sedentary conditions with 30 min of moderate walking. Exclusion of walking would decrease the PAL to 1.29, which represents a minimal survival level of physical activity. The mean free-living PAL of our women, as determined by DLW measurements, was 1.86. Assigning a multiple of 1.60 to these women would underestimate their energy requirements by an average of 1109 kJ/d.

Although significant interindividual variation in TEE was observed, TEE may be used to estimate the energy intakes required to sustain the lifestyles of moderately active women of reproductive age in industrialized societies. On the basis of TEE, current recommended energy intakes for healthy, moderately active women of reproductive age living in industrialized societies should be revised.

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NFB was responsible for the study design and analysis and for writing the manuscript; MST and JMH were responsible for data collection; WWW, NRM, and EOS were responsible for sample analysis; and EOS was responsible for the statistical analysis. None of the authors had any financial or personal affiliation with any company or organization that sponsored this research.

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