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The Influence of Body Composition and Lifestyle Factors on Resting Energy Expenditure and Its Role in Cardiometabolic Risk: A Cross-Sectional Study

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Abstract: In this study, we investigated the associations between lifestyle factors (diet, physical activity, sleep), body composition, and resting energy expenditure (REE) in a cohort of 77 healthy, non-obese adults aged 30–45 years. Using indirect calorimetry, accelerometers, and bioelectrical impedance analysis (BIA), we assessed REE, physical activity levels, sleep duration, and biochemical parameters to identify factors contributing to individual variations in REE and their potential role in modulating cardiometabolic risk.We found that fat-free mass (FFM) was the strongest predictor of REE, along with related metrics such as total body water, body cell mass, and muscle mass ($p < 0.0001$, adj. $R^2 > 0.5$). In univariable models, all physical activity intensities were significantly associated with REE, but only moderate physical activity (MPA) remained significant after adjusting for sex and FFM ($\beta = 2.2 \pm 1.0$, $p < 0.05$, adj. $R^2 = 0.589$). Similarly, a positive association between HDL-C and REE persisted after adjustments ($\beta = 5.0 \pm 2.0$ kcal/d, $p < 0.05$, adj. $R^2 = 0.588$). These direct links may be attributed to habitual, spontaneous physical activity, which generates post-exercise metabolic elevation and promotes adipose tissue browning, resulting in favorable metabolic effects.Other biochemical and lifestyle factors, including HOMA-IR, insulin levels, triglycerides, and total energy intake, showed positive associations with REE in the crude model. However, these relationships diminished after adjustment, suggesting that their influence is likely mediated by factors such as body composition, body size, and sex. Finally, no significant relationship between sleep and REE was observed in our cohort under naturalistic conditions, possibly due to the alignment of participants' sleep durations with recommended guidelines.

Keywords: resting energy expenditure; indirect calorimetry; body composition; biochemical markers; physical activity; total sleep time; diet; accelerometers; cardiometabolic risk

1. Introduction

Total daily energy expenditure (TEE) consists of three main components: resting energy expenditure (REE), the thermic effect of food, and energy expenditure from physical activity. REE, defined as the minimal metabolic rate necessary to sustain life, includes the energy required for essential organ functions at rest and typically accounts for 60–70% of TEE in most individuals [1,2]. Among the various methods to assess REE, indirect calorimetry (IC) is considered the "gold standard" [3]. IC determines energy expenditure by measuring a subject's oxygen consumption (VO_2) and carbon dioxide production (VCO_2). The respiratory quotient (RQ), defined as the ratio of exhaled CO_2 to consumed O_2, serves as the basis for interpreting REE results [4]. This method enables precise determination of basal nutritional requirements, providing valuable insights for personalized nutritional planning [2]. However, the high costs of specialized equipment and the need for trained clinical staff limit the widespread application of IC. Consequently, various equations have been developed to estimate REE in clinical practice [5–7]. These equations typically incorporate gender, age, weight, and height [5,8,9], although numerous other factors—such as body composition,

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ethnicity, physical activity (PA), hormonal status, biochemical parameters, genetics, and lifestyle—contribute to individual variability in REE [10–12].

In this study, we explored how lifestyle factors (diet, physical activity, sleep), body composition, and biochemical parameters influence resting energy expenditure (REE) in a group of 77 healthy, non-obese adults aged 30–45 years. By employing indirect calorimetry, accelerometers, and bioelectrical impedance analysis (BIA), we evaluated REE alongside physical activity levels, sleep duration, and key biochemical markers to uncover contributors to individual variability in REE and their potential impact on cardiometabolic risk.

2. Materials and Methods

2.1. Subjects and Data Collection

This cross-sectional study was conducted at the Medical University of Warsaw with 77 healthy, non-obese adults (31 men and 46 women) recruited through advertisements. The inclusion criteria were age 30–45 years, no diagnosed chronic diseases, and a BMI between 18.5 kg/m² and 29.9 kg/m². Exclusion criteria included pharmacological treatment and contraindications for body composition analysis, such as epilepsy, implanted cardiac pacemakers, defibrillators, or metal endoprostheses. Body weight and height were measured using a Seca 799 measurement station and column scales with a precision of ±0.1 kg/cm. Waist circumference was measured with a steel measuring tape, positioned midway between the lower border of the ribs and the iliac crest in the horizontal plane. The study protocol was approved by the Ethics Committee of the Medical University of Warsaw (KB/158/2021), and all participants provided written informed consent.

2.2. Resting Energy Expenditure Measured By Indirect Calorimetry

REE was measured using indirect calorimetry (Q-NRG+, COSMED Srl, Rome, Italy) in oronasal face mask mode with an external turbine flowmeter. The flowmeter and sampling line were connected to the mask. REE was calculated based on the measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2), along with other ventilatory parameters. Prior to each test session, the instrument was warmed up and calibrated automatically. As per the manufacturer's instructions, two-point gas analyzer calibration was performed monthly, along with turbine calibration using a calibration syringe. All tests were conducted in the morning (08:30–11:00 a.m.) after a 12-hour fast and 12-hour avoidance of strenuous exercise. Participants were instructed to avoid speaking and to relax without falling asleep during the test. Data were collected over a 20-minute interval, with the first 5 minutes used for familiarization and excluded from analysis. The VO_2 and VCO_2 values were used to calculate the respiratory quotient (RQ) as the ratio of VCO_2/VO_2, and REE (in kcal/day) was derived using the Weir equation [13].

2.3. Bioelectrical Impedance Analysis, Biochemical Tests, Physical Activity, and Sleep Duration

The methods for bioelectrical impedance analysis, biochemical tests, physical activity measurement, and sleep duration assessment were described in detail in our previous publication (Ostrowska et al., Nutrients 2024, 16, 266) [14]. Briefly, body composition was assessed using bioelectrical impedance analysis (Bioscan 920-2, Maltron Int, UK), following ESPEN guidelines. Biochemical tests included fasting serum measurements of insulin, glucose, lipid profiles, and high-sensitivity C-reactive protein. The Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated based on fasting insulin and glucose concentrations. Physical activity and sleep duration were monitored over 7 days using wGT3X-BT ActiGraph accelerometers (ActiGraph LLC, Pensacola, FL, USA), with physical activity intensity classified using Freedson's cut-offs.

2.4. Statistical Analyzes

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Descriptive statistics were used to summarize the collected data. Quantitative variables were presented as means and standard deviations after assessing their distribution with the Shapiro-Wilk test. Comparisons of continuous variables between males and females were conducted using the independent samples t-test. To evaluate the influence of various factors on REE, univariable linear regression analysis was performed (crude model). These models were subsequently adjusted for sex, and fat-free mass (FFM) (adjusted model, Model 1). The adjusted coefficient of determination (adj. R^2) and p-value were used to assess the predictive power of each factor. Prior to conducting the regression analysis in Model 1, the linearity of each relationship was verified. Statistical significance was set at $p < 0.05$. All analyses were conducted using R statistical software, version 3.4.0 (R Core Team, 2017)

3. Results

The study included 77 participants (31 men and 46 women) with a mean age of 37 years, average weight of 72 kg, height of 173 cm, and BMI of 24 kg/m². No significant differences in age or percentage of fat-free mass (FFM) were observed between males and females ($p > 0.05$). However, men had significantly higher absolute FFM (63 vs. 44 kg, $p \leq 0.001$) and a lower average percentage of fat mass (26% vs. 29%, $p < 0.05$) compared to women. Other body composition components also showed significant differences between sexes ($p < 0.05$).

Regarding metabolic markers, no sex-specific differences were found in HOMA-IR, total cholesterol (TC), LDL-C, and CRP levels. However, men had significantly higher triglyceride (TG) levels (118 vs. 78 mg/dL, $p < 0.001$) and lower HDL-C levels (53 vs. 67 mg/dL, $p < 0.001$) compared to women. In terms of lifestyle factors, men engaged in significantly more physical activity (MVPA, including both moderate and vigorous PA) than women (91 vs. 59 min/day, $p < 0.05$). Dietary intake also varied by sex, with men reporting higher energy, protein, and fat intake, while no differences in sleep duration were observed between sexes. Descriptive characteristics of the study population are summarized in Table 1.

Table 1. Characteristic of study participants.

	Total, *n* = 77		**Females, *n* = 46**		**Males, *n* = 31**		
Basic parameters	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**	***p*-value**
Age (years)	37 (4.7)	28.0, 45.0	36 (4.4)	28.0, 45.0	38 (5.1)	30.0, 45.0	ns.
Body weight (kg)	72 (14.3)	44.0, 107.0	63 (8.1)	44.0, 82.0	85 (10.4)	57.0, 107.0	< 0.001
Height (cm)	173 (9.6)	150.0, 194.0	167 (6.4)	150.0, 178.0	182 (5.9)	171.0, 194.0	< 0.001
BMI (kg/m²)	24 (3.1)	18.6, 29.5	23 (2.5)	18.6, 28.1	26 (2.9)	18.6, 29.5	< 0.001
WC (cm)	84 (11.6)	63.0, 110.0	78 (8.1)	63.0, 91.0	93 (9.5)	65.0, 110.0	< 0.001
Body composition parameters							
FFM (kg)	52 (10.3)	34.3, 75.0	44 (3.6)	34.3, 51.2	63 (5.8)	46.2, 75.0	< 0.001
FFM (%)	71 (9.8)	0.7, 84.3	71 (5.7)	60.8, 83.6	72 (14.0)	0.7, 84.3	ns.
FAT (kg)	20 (6.5)	8.8, 42.2	19 (5.6)	9.7, 32.1	23 (6.9)	8.8, 42.2	< 0.01
FAT (%)	28 (5.5)	15.7, 39.2	29 (5.5)	19.8, 39.2	26 (5.1)	15.7, 38.4	< 0.05
VAT (cm²)	118 (82.0)	21.0, 350.0	84 (50.2)	30.0, 276.0	171 (92.7)	21.0, 350.0	< 0.001
SAT (cm²)	97 (35.0)	28.0, 201.0	88 (32.3)	28.0, 201.0	110 (35.5)	46.0, 173.0	< 0.05
VAT/SAT	1 (0.6)	0.3, 2.9	1 (0.3)	0.3, 2.0	2 (0.7)	0.5, 2.9	< 0.001
TBW (Lt)	37 (7.8)	24.0, 54.5	31 (2.9)	24.0, 36.9	46 (4.1)	34.6, 54.5	< 0.001
TBW (%)	51 (3.6)	41.9, 62.9	50 (3.1)	41.9, 57.0	53 (3.4)	46.6, 62.9	< 0.001

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ECW (Lt)	17 (2.7)	12.2, 22.8	15 (1.5)	12.2, 19.3	19 (1.7)	14.9, 22.8	< 0.001
ECW (%)	45 (7.5)	0.4, 69.8	48 (6.4)	15.5, 69.8	41 (7.6)	0.4, 46.6	< 0.001
ICW (l)	21 (6.2)	7.3, 47.7	17 (5.1)	7.3, 47.7	26 (2.6)	19.2, 31.9	< 0.001
ICW (%)	54 (4.4)	30.2, 59.0	52 (4.3)	30.2, 56.2	57 (1.2)	53.4, 59.0	< 0.001
ECW/ICW	1 (0.2)	0.7, 2.3	1 (0.2)	0.8, 2.3	1 (0.0)	0.7, 0.9	< 0.001
BCM (kg)	27 (6.2)	14.1, 41.1	23 (2.3)	14.1, 26.9	34 (3.1)	25.1, 41.1	< 0.001
ECM (kg)	25 (4.3)	17.4, 34.6	22 (1.8)	17.4, 25.9	29 (2.8)	21.1, 34.6	< 0.001
Protein mass (kg)	11 (2.4)	6.6, 18.2	9 (1.1)	6.6, 11.5	13 (2.1)	8.6, 18.2	< 0.001
Mineral mass (kg)	4 (0.7)	2.7, 6.4	4 (0.4)	2.7, 4.7	5 (0.7)	3.0, 6.4	< 0.001
Muscle mass (kg)	24 (6.5)	12.6, 37.8	19 (1.8)	12.6, 22.6	31 (3.2)	22.8, 37.8	< 0.001
TBK (gr)	125 (32.9)	63.5, 196.3	101 (10.3)	63.5, 119.0	162 (15.7)	118.4, 196.3	< 0.001
TBCa (gr)	1,031 (238.1)	582.0, 1,543.0	852 (74.2)	582.0, 984.0	1,298 (113.4)	979.0, 1,543.0	< 0.001
Glycogen mass (gr)	469 (93.5)	311.0, 682.0	401 (34.5)	311.0, 465.0	571 (50.6)	420.0, 682.0	< 0.001
Dry weight (kg)	70 (14.7)	38.2, 108.3	61 (8.7)	38.2, 78.7	84 (10.7)	53.3, 108.3	< 0.001
Biochemical parameters							
TC (mg/dl)	199 (30.3)	107.3, 268.8	198 (26.9)	150.9, 262.4	200 (35.2)	107.3, 268.8	ns.
HDL-C (mg/dl)	61 (14.3)	35.2, 105.0	67 (14.4)	44.2, 105.0	53 (9.4)	35.2, 76.0	< 0.001
LDL-C (mg/dl)	119 (24.0)	62.0, 190.0	115 (21.7)	62.0, 170.0	125 (26.4)	93.0, 190.0	ns.
TG (mg/dl)	94 (47.2)	35.3, 340.0	78 (26.4)	38.2, 144.9	118 (60.3)	35.3, 340.0	< 0.001
Fasting blood glucose (mg/dl)	97 (7.3)	80.0, 118.0	97 (5.9)	82.0, 111.0	99 (8.9)	80.0, 118.0	ns.
Fasting insulin (μU/ml)	8 (4.5)	2.2, 25.0	7 (2.9)	2.8, 14.2	10 (5.9)	2.2, 25.0	ns.
HOMA-IR	2 (1.2)	0.5, 6.4	2 (0.7)	0.6, 3.4	2 (1.6)	0.5, 6.4	ns.
CRP (mg/l)	1 (2.8)	0, 24.0	1 (2.0)	0, 5.8	2 (4.2)	0, 24.0	ns.
Indirect calorimetry parameters							
VO2 (ml/min)	249 (53.6)	155.8, 370.7	220 (34.9)	155.8, 297.4	294 (45.2)	191.9, 370.7	< 0.001
VCO2 (ml/min)	231 (58.8)	135.4, 468.3	201 (34.5)	135.4, 299.0	276 (59.1)	190.3, 468.3	< 0.001
RQ factor	1 (0.1)	0.8, 1.3	1 (0.1)	0.8, 1.1	1 (0.1)	0.8, 1.3	ns.
REE (kcal/day)	1,766 (389.0)	1,089.7, 2,823.6	1,552 (246.5)	1,089.7, 2,120.8	2,087 (340.2)	1,384.4, 2,823.6	< 0.001
Physical activity and sleep parameters							
MPA (min/day)	63 (31.8)	22.8, 183.0	54 (18.9)	22.8, 100.1	77 (41.6)	30.3, 183.0	< 0.05
VPA (min/day)	8 (14.7)	0.0, 60.0	5 (7.3)	0.1, 31.6	14 (20.4)	0.0, 60.0	< 0.05
MVPA (min/day)	72 (43.5)	23.6, 236.5	59 (21.2)	23.6, 114.5	91 (59.4)	34.3, 236.5	< 0.05
TST (min/night)	455 (58.2)	289.0, 609.0	458 (69.0)	289.0, 609.0	451 (35.8)	387.1, 518.2	ns.
Diet parameters							

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Energy (kcal/d)	2038.99 (455.75)	1287.0, 3132.4	1797.06 (271.54)	1287.0, 2526.3	2406.37 (435.13)	1447.3, 3132.4	<0.001
Protein (g/d)	85.44 (24.31)	28.0, 137.2	72.97 (16.57)	28.0, 102.3	104.38 (21.95)	76.7, 137.2	<0.001
Fats (g/d)	77.65 (21.42)	43.8, 150.8	70.18 (15.19)	43.8, 108.0	89.00 (24.60)	48.4, 150.8	<0.01
Carbohydrates (g/d)	242.83 (65.02)	125.9, 390.6	219.68 (39.39)	128.4, 311.0	277.99 (79.87)	125.9, 390.6	<0.01

Abbreviations: BMI - body mass index, WC - waist circumference, FFM - fat free mass, FM - fat mass, VAT – visceral adipose tissue, SAT - subcutaneous adipose tissue, TBW - total body water, ECW - extracellular water, ICW - intracellular water, BCM - body cell mass, ECM - extracellular mass, TBK - total body potassium, TBCa - total body calcium, HOMA-IR -homeostatic model assessment insulin resistance, TC - total cholesterol, TG – triglycerides, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, CRP - c-reactive protein, VO2 - volume oxygen, VCO2 - volume carbon dioxide, RQ - respiratory quotient, REE - resting energy expenditure, MPA - moderate physical activity, VPA - vigorous physical activity, MVPA – moderate and vigorous physical activity, TST - total sleep time

3.1. Impact of Anthropometric and Body Composition Factors on REE

Table 2 summarizes the influence of anthropometric and body composition parameters on REE. Among the basic parameters, body weight, height, BMI, and waist circumference showed strong associations with REE, with body weight having the highest predictive power in this group ($\beta = 19.8 \pm 2.1$ kcal/d, $p < 0.0001$, adj. $R^2 = 0.523$). Age did not have a significant impact on REE, likely due to the deliberate selection of participants within a similar age range.

In the body composition parameters, measures related to fat-free mass (FFM), including protein mass, muscle mass, dry weight, body cell mass (BCM), and water compartments (total body water [TBW], extracellular water [ECW], and intracellular water [ICW]), demonstrated strong associations with REE, consistent with the high energy demands of metabolically active tissue. Among these, FFM ($\beta = 28.8 \pm 2.8$ kcal/d, $p < 0.0001$, adj. $R^2 = 0.572$) was the most substantial predictor, reinforcing the role of lean mass in determining resting energy requirements.

In contrast, fat mass (FM) showed the lowest predictive power for REE among body composition parameters ($p < 0.001$, adj. $R^2 = 0.171$). This effect was especially weak for subcutaneous adipose tissue (SAT), with subcutaneous fat area demonstrating minimal association with REE ($p < 0.05$, adj. $R^2 = 0.066$). Visceral adipose tissue (VAT), however, exhibited a slightly stronger association with REE, reflecting its higher metabolic activity compared to SAT.

Table 2. Influence of anthropometric and body composition components on REE.

Basic parameters	***p* – value**	**β ± SE**	**95%Cl**	**Adj. R^2**
Age (years)	Ns.	-	-	-
Sex	< 0.0001	535.7 ± 65.6	405.0, 666.2	0.454
Body weight (kg)	< 0.0001	19.8 ± 2.1	15.6, 24.1	0.523
Height (cm)	< 0.0001	26.8 ± 3.4	20.0, 33.7	0.427
BMI (kg/m²)	< 0.0001	68.6 ± 11.9	45.0, 92.3	0.291
WC (cm)	< 0.0001	22.1 ± 2.9	16.4, 27.8	0.426
Body composition parameters				
FFM (kg)	< 0.0001	28.8 ± 2.8	23.2, 34.3	0.572
FAT (kg)	< 0.001	25.5 ± 6.1	13.3, 37.8	0.171
VAT (cm²)	< 0.0001	2.5 ± 0.5	1.6, 3.4	0.264
SAT (cm²)	< 0.05	3.0 ± 1.2	0.7, 5.5	0.066
VAT/SAT	< 0.0001	366.3 ± 63.6	239.7, 492.9	0.289

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TBW (Lt)	< 0.0001	37.2 ± 3.7	29.8, 44.6	0.556
ECW (Lt)	< 0.0001	100.7 ± 12.0	76.7, 124.7	0.466
ICW (Lt)	< 0.0001	38.7 ± 5.6	27.6, 49,7	0.375
BCM (kg)	< 0.0001	47.4 ± 4.6	38.2, 56.6	0.570
ECM (kg)	< 0.0001	66.9 ± 7.0	53.0, 80,8	0.535
Protein mass (kg)	< 0.0001	106.7 ± 13.7	79.3, 134.1	0.429
Muscle mass (kg)	< 0.0001	44.8 ± 4.5	35.8, 53.7	0.554
Dry weight (kg)	< 0.0001	19.1 ± 2.1	15.0, 23.3	0.516

Abbreviations: REE - resting energy expenditure, Adj. R^2 - the adjusted coefficient of determination, β ± SE - regression coefficient ± standard error, Cl - confidence interval, BMI - body mass index, WC - waist circumference, FFM - fat free mass, FM - fat mass, VAT – visceral adipose tissue, SAT - subcutaneous adipose tissue, TBW - total body water, ECW - extracellular water, ICW - intracellular water, BCM - body cell mass, ECM - extracellular mass, TBK - total body potassium, TBCa - total body calcium.

3.2. Impact of Biochemical Factors on REE

Regarding biochemical factors, in the crude model, a significant inverse relationship was found between REE and HDL-C (β = -7.0 ± 3.0 kcal/d, $p < 0.05$, adj. $R^2 = 0.053$). Positive significant associations were observed for TG (β = 3.2 ± 0.9 kcal/d, $p < 0.001$, adj. $R^2 = 0.142$), HOMA-IR (β = 89 ± 35.0 kcal/d, $p < 0.05$, adj. $R^2 = 0.065$), and fasting insulin levels (β = 24 ± 9.3 kcal/d, $p < 0.05$, adj. $R^2 = 0.067$). After adjustment for sex and FFM in Model 1, the significant associations of REE with TG, HOMA-IR, and insulin levels were no longer present. Notably, the association with HDL-C remained significant in Model 1, but the relationship became positive instead of inverse (β = 5.0 ± 2.0 kcal/d, $p < 0.05$, adj. $R^2 = 0.588$). These results suggest that the relationship between HDL-C and REE may be more robust, while associations with TG, HOMA-IR, and insulin are likely influenced by body composition and demographic factors (Table 3).

Table 3. Influence of biochemical parameters on REE – crude model and model adjusted for sex and FFM (kg).

	Crude model				**Model 1 Adjusted for sex, FFM**			
Biochemical parameters	*p*-value	β ± SE	95%Cl	Adj. R^2	p-value	β ± SE	95%Cl	Adj. R^2
TC (mg/dl)	ns.	-	-	-	ns.	-	-	-
HDL-C (mg/dl)	< 0.05	- 7.0 ± 3.0	- 13.0, -1.1	0.053	< 0.05	5.0 ± 2.0	1.1, 9.0	0.588
LDL-C (mg/dl)	ns.	-	-	-	ns.	-	-	-
TG (mg/dl)	< 0.001	3.2 ± 0.9	1.5, 5.0	0.142	ns.	-	-	-
CRP (mg/l)	ns.	-	-	-	ns.	-	-	-
Fasting blood glucose (mg/dl)	ns.	-	-	-	ns.	-	-	-
Fasting insulin (μU/ml)	< 0.05	24 ± 9.3	5.5, 43,0	0.067	ns.	-	-	-
HOMA-IR	< 0.05	89 ± 35.0	19.5, 158.5	0.065	ns.	-	-	-

Abbreviations: REE - resting energy expenditure, Adj. R^2 - the adjusted coefficient of determination, β ± SE - regression coefficient ± standard error, Cl - confidence interval, HOMA-IR -homeostatic model assessment insulin resistance, TC - total cholesterol, TG – triglycerides, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, CRP - c-reactive protein.

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3.3. Impact of lifestyle factors (physical activity, sleep, diet) on REE

The table presents linear regression models evaluating lifestyle predictors of resting energy expenditure (REE). All levels of physical activity intensity were significantly associated with REE in the crude model. However, after adjusting for sex, and FFM, the association with vigorous physical activity (VPA) was no longer significant. Moderate physical activity (MPA) and moderate-to-vigorous physical activity (MVPA) retained significant associations with REE in the adjusted model (MPA: $\beta = 2.2 \pm 1.0$, $p < 0.05$, adj. $R^2 = 0.589$; MVPA: $\beta = 1.5 \pm 0.7$, $p < 0.05$, adj. $R^2 = 0.590$). Sleep duration showed no significant association with REE in either the crude or adjusted models.

For dietary intake, Energy was the only significant predictor of REE in the crude model ($\beta = 0.7 \pm 0.2$, $p < 0.01$; adj. $R^2 = 0.218$). After adjusting for sex and FFM, Energy remained the main predictor, though the association approached but did not reach statistical significance ($p = 0.06$). Protein, Fats, and Carbohydrates showed minimal and non-significant associations with REE in both models (Table 3).

Table 4. Influence of lifestyle factors on REE – crude model and model adjusted for sex and FFM (kg).

	Crude model				**Model 1 Adjusted for sex, FFM**			
Physical activity and sleep parameters	*p*-value	β ± SE	95%Cl	Adj. R^2	p-value	β ± SE	95%Cl	Adj. R^2
MPA (min/day)	< 0.0001	5.7 ± 1.2	3.2, 8.1	0.204	< 0.05	2.2 ± 1.0	0.2, 4.1	0.589
VPA (min/day)	< 0.0001	11.0 ± 3.0	5.1, 16.3	0.190	ns.	-	-	-
MVPA (min/day)	< 0.001	4.3 ± 0.9	2.5, 6.0	0.221	< 0.05	1.5± 0.7	0.1, 3.0	0.590
TST (min/night)	ns.	-	-	-	ns.	-	-	-
Diet parameters	p-value	β ± SE	95%Cl	Adj. R^2	p-value	β ± SE	95%Cl	Adj. R^2
Energy (kcal/d)	<0.01	0.7±0.2	0.3,1.2	0.218	ns (0.06)	-	-	-
Protein (g/d)	ns	-	-	-	ns	-	-	-
Fats (g/d)	ns	-	-	-	ns	-	-	-
Carbohydrates (g/d)	ns	-	-	-	ns	-	-	-

Abbreviations: REE - resting energy expenditure, Adj. R^2 - The adjusted coefficient of determination, β ± SE - regression coefficient ± standard error, Cl - confidence interval, MPA - moderate physical activity, VPA - vigorous physical activity, MVPA – moderate and vigorous physical activity, TST - total sleep time.

4. Discussion

In this cross-sectional study, we aimed to investigate the associations between lifestyle factors, body composition, and Resting Energy Expenditure (REE). Additionally, we examined the potential role of REE in modulating cardiometabolic risk through its interactions with biochemical and anthropometric parameters. To the best of our knowledge, no previous studies have comprehensively focused on the anthropometric, biochemical, and lifestyle factors contributing to individual variations in REE among healthy, non-obese individuals. Furthermore, the study employed advanced techniques to assess body composition (multi-frequency bioelectrical impedance), resting energy expenditure (indirect calorimetry), and physical activity and sleep quality (tri-axial accelerometers). These techniques represent an objective approach to data collection and offer significant advantages over self-reported or estimated methodologies.

The results of our study align with findings emphasizing the predictive value of BIA-derived variables for resting energy expenditure (REE) [10,18,19]. Fat-free mass (FFM), alongside components like total body water, body cell mass (BCM), and muscle mass, demonstrated the highest predictive power for REE (e.g., FFM: adj. $R^2 = 0.572$, $p < 0.0001$) [10]. These variables, linked to metabolically active tissues, outperform basic anthropometric parameters such as body weight, height, and BMI [18]. Interestingly, fat mass (FM) showed a significantly weaker association with REE (adj. $R^2 = 0.171$,

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$p < 0.001$), particularly subcutaneous adipose tissue (SAT), which had minimal predictive value (adj. $R^2 = 0.066$, $p < 0.05$) compared to visceral adipose tissue (VAT) (adj. $R^2 = 0.264$, $p < 0.0001$) [19]. This discrepancy is likely due to SAT's lower metabolic activity and distinctive gene expression patterns, including higher adiponectin and reduced proinflammatory adipokine expression [20,21]. These findings reinforce the utility of BIA metrics as robust predictors of REE and underscore the nuanced role of different fat depots in energy metabolism.

Another aspect is the immunological function, which accounts for up to 15% of daily energy expenditure [22]. In clinical trials, C-reactive protein (CRP) has been identified as a biomarker reflecting inflammation [22,23]. Most studies exploring the potential association between REE and CRP have focused on severely ill patients, such as those with pancreatic cancer [24], sepsis [25], or chronic kidney disease [26]. However, in our study, no significant association was found between REE and inflammation measured by CRP in a cohort of healthy normal-weight and overweight individuals. On the other hand, a positive association between REE and HOMA-IR was initially observed. This relationship (HOMA-IR and REE), reported in previous studies [22,27,28], was not replicated in our study after adjustment (Table 3, Model 1). This finding supports the hypothesis that the observed dependence is primarily due to increased body size. In our study, we noted a significant positive correlation between fat-free mass (FFM), a component of Model 1, and body weight ($r = 0.9$, $p < 0.0001$). Consequently, increased body mass is associated with elevated oxidative stress and a higher production of proinflammatory cytokines. Chronic inflammation, in turn, induces insulin dysfunction [22], which may explain elevated insulin levels and HOMA-IR. A similar pattern was observed for triglyceride (TG) concentrations, where the crude model showed a significant association between REE and TG ($\beta = 3.2 \pm 0.9$, $p < 0.001$, adj. $R^2 = 0.142$). However, this association disappeared after adjustment in Model 1. Interestingly, a significant link between REE and HDL-C persisted even after adjustment. While the crude model revealed an inverse association between HDL-C and REE, a proportional relationship emerged post-adjustment (Table 3). This direct link ($p < 0.05$) with REE, observed after adjustment, was also noted for moderate physical activity (MPA) (Table 4, Model 1). Typically, changes in physical activity levels influence energy balance, which should result in alterations in body mass or composition [29]. This relationship between physical activity (PA), FFM, and REE is particularly relevant for vigorous physical activity (VPA) [30]. Therefore, after adjustment, the previously mentioned relationship disappeared (Table 4, Model 1). In contrast, the association between MPA and REE in both adjusted and unadjusted models can be attributed to habitual, spontaneous physical activity, which occurs more frequently than planned training sessions. Furthermore, PA contributes to post-exercise elevation of metabolic rate, with REE remaining elevated as long as PA is performed regularly, at least three times per week [35]. Additionally, exercise or diet can stimulate the browning of adipose tissue (AT), leading to favorable local and systemic metabolic effects [20]. This process of exercise-induced AT browning may partly explain the beneficial effects of healthy lifestyle factors on the systemic metabolic state, which are evident in both adjusted and unadjusted models. Moreover, evidence suggests that the coupling between energy expenditure and energy intake is weaker at low and moderate levels of physical activity than during vigorous physical activity [31]. Regular exercise has also been shown to increase high-density lipoprotein (HDL) cholesterol concentrations [32], which could explain the observed relationship between HDL-C and REE in the adjusted model.

Efficient sleep duration is increasingly recognized as a critical factor in maintaining normal body weight [33]. Epidemiological studies have reported an inverse or U-shaped relationship between sleep duration and weight [33,34]. However, the relationship between sleep and REE remains less well characterized. Some studies have found that decreased sleep time is associated with increased respiratory quotient (RQ) and REE [33,35,36], while others have reported no significant effect [37,38], which aligns with the findings of our study (Table 4). These discrepancies may stem from variations in participant characteristics and study designs - for instance, a cross-sectional study on 126 obese individuals who regularly slept less than 6.5 hours per night found that poor sleep quality was associated with increased REE, likely due to stress system activation. This study estimated that a 10-

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μg/dl increase in serum cortisol could elevate REE by approximately 10% [33]. Similarly, in a study of healthy lean participants, experimentally induced total sleep deprivation and sleep fragmentation led to an approximate 7% increase in energy expenditure [39]. In contrast, our study was conducted under naturalistic conditions, with participants' mean total sleep time aligning with the National Sleep Foundation's recommendations of around 7.5 hours per night (Table 1) [40]. These naturalistic conditions likely influenced the outcomes, minimizing the impact of sleep deprivation on REE.

Other lifestyle factors, such as diet, also play a role in modulating REE. Higher protein intake has been associated with increased REE; for example, a 1% increase in protein intake can raise REE by approximately 3 kcal/day [41]. Additionally, high-protein diets are well-documented for their role in preserving fat-free mass (FFM) and maintaining REE during weight loss [42]. However, this relationship was not confirmed in our study. In the crude model, energy intake significantly predicted REE ($\beta = 0.717$, $p = 0.0027$), whereas protein intake demonstrated only a marginal and non-significant effect. After adjustment, the predictive power of energy intake on REE was further reduced ($\beta = 0.376$, $p = 0.0630$), indicating that the impact of energy on REE may be mediated by factors such as sex and changes in body composition.

Some limitations of the present study should be considered when interpreting the findings. While the accelerometers used are reliable for capturing overall activity patterns, they have lower sensitivity to sedentary behaviors and are unable to effectively register static exercises or physical activities that do not involve a transfer of the center of mass, such as carrying a load [43]. Additionally, the female participants were examined without accounting for their menstrual cycle phases, which can influence several physiological parameters, including REE and sleep, potentially introducing minor variability [44].

5. Conclusions

This study provides novel insights into the associations between lifestyle factors, body composition, and resting energy expenditure (REE) in a cohort of healthy, non-obese individuals. By employing advanced techniques such as multi-frequency bioelectrical impedance, indirect calorimetry, and tri-axial accelerometers, we offered an objective and comprehensive approach to examining the determinants of REE. Our findings reinforce the central role of fat-free mass (FFM) and related body composition metrics as the most robust predictors of REE, surpassing traditional anthropometric measures such as BMI and body weight.

While energy intake demonstrated a significant association with REE in the crude model, this relationship diminished after adjustment, suggesting that its influence is likely mediated by factors such as body composition and sex. Interestingly, associations between protein intake and REE, well-supported in the literature, were not confirmed in this study. Similarly, the relationship between sleep and REE was not demonstrated in our cohort under naturalistic conditions, possibly due to the alignment of participants' sleep durations with recommended guidelines.

In contrast, our findings revealed a significant potential link between moderate physical activity (MPA) and REE, with particular emphasis on frequently undertaken moderate physical activity and its associated increase in HDL-C concentrations. These potential direct links between MPA–REE and HDL–REE could be explained by habitual, spontaneous physical activity, which is carried out much more often than planned training. Physical activity generates a post-exercise elevation of metabolic rate and contributes to the browning of adipose tissue, leading to favorable local and systemic metabolic effects.

Future research should aim to further explore these relationships in larger, more diverse populations and under controlled conditions to enhance our understanding of the mechanisms driving energy expenditure and its broader implications for cardiometabolic health.

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