Original Article

Resting Energy Expenditure in Normal-Weight and Overweight/Obese Subjects Was Similar Despite Elevated Sympathovagal Balance

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Key Words

Autonomic nervous system • Energy expenditure • Fat-free mass • Heart rate variability • Obesity

Abstract

Objective: Alterations in the autonomic nervous system (ANS) may cause impairment in the metabolic processes that can lead to weight gain. The purpose of this study was to determine the difference between the resting energy expenditure (REE) and the resting ANS activity in overweight/obese and normal-weight healthy subjects. **Method:** Group 1 consisted of 18 subjects with BMI > 25 kg/m², and 20 subjects with BMI ranging from 20 to 25 kg/m² formed group 2. Measurements of low-frequency (LF) and high-frequency (HF) power components expressed in normalized units (LFnu, HFnu) and LF/HF ratio were assessed for analysis of heart rate variability, and simultaneously REE measurement was performed. **Results:** The mean LFnu (27.2% increased) and the LF/HF were higher and the mean HFnu was lower (29.9% decreased) in group 1 than in group 2 ($p < 0.01$). Although a statistical difference was observed in REE between groups, REE per kilogram corrected for fat-free mass ($REE_{FFMcorr}$) was 21.47 ± 2.92 kcal/day/kg in group 1, and 21.56 ± 1.90 kcal/day/kg in group 2, and this difference was not statistically significant ($p > 0.05$). **Conclusion:** We concluded that $REE_{FFMcorr}$ in overweight/obese and normal-weight subjects were similar despite elevated sympathovagal balance in overweight/obese individuals, and $REE_{FFMcorr}$ should be used to avoid misestimating the REE in obese and normal-weight subjects.

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Introduction

Obesity and overweight are the major risk factors for metabolic and cardiovascular diseases, and their prevalence is increasing throughout the world [1]. Eating disorders and sedentary lifestyle are the main reasons for gaining weight, but in some cases genetically mediated metabolic properties or disorders can be the cause of obesity [2–4]. The autonomic nervous system (ANS) plays a vital role in the metabolic processes of homeostatic regulation in the body. The alterations in the ANS result in impairment in the metabolic processes that can lead to weight gain [5].

The term heart rate variability (HRV) refers to variations in both heart rate and RR intervals [6]. Sinoatrial node, which determines the normal rhythm of the heart, is regulated by innervations from both the sympathetic and parasympathetic components of the ANS [7]. HRV analysis is used for the assessment of autonomic cardiac activity that may enable the evaluation of the central regulation and interaction of sympathetic and parasympathetic system activity [5–7]. Cardiac sympathetic and parasympathetic activities, high-frequency (HF), low-frequency (LF) components of HRV, and their ratio can be determined by power spectral analysis of HRV [8].

Obesity may alter the balance between the sympathetic and parasympathetic nervous system [9]. There is no consensus about the relationship between obesity and ANS activity level. The opposite hypothesis that most obese subjects are low in sympathetic activity (the MONA LISA hypothesis) [10] was also confirmed by some studies [9, 11].

Determination of the energy expenditure by indirect calorimetry is a gold standard method in the clinical practice [12]. The resting energy expenditure (REE) measurement has been used in the obesity-related research to assess the balance between energy intake and energy expenditure [13]. Estimation of energy expenditure is also required in designing a weight loss program. Exploring the relationship between REE and ANS in obese subjects may have clinical impact in optimizing weight loss and maintenance programs, and especially for pharmacological interventions aiming at a stimulation of the sympathetic nervous system (SNS) activity.

This study was designed to determine whether or not there is a difference between REE and the resting ANS activity in overweight/obese and normal weight healthy subjects.

Material and Methods

Subjects

16 female and 22 male Caucasian normotensive healthy volunteers between the ages of 17–45 years (mean age = 29.24 ± 7.16 years; mean height = 169.32 ± 10.48 cm; mean mass = 74.34 ± 18.07 kg) were recruited for the study. All participants were healthy non-smokers and were free of physical illness which can be detected by a physical examination. Their weights were stable for 6 months (± 2 kg), and none of them was on a diet to lose weight. The subjects were not regularly taking any medications, and subjects taking medications that might have influenced HRV and REE were excluded from the study. Participants were classified in two groups based on their BMI. Group 1 consisted of 18 subjects with BMI > 25 kg/m², and 20 subjects with BMI ranging from 20 to 25 kg/m² formed group 2. Ethical approval was taken from the local ethical committee, and an informed consent was also obtained from all participants. All procedures used in this study were in agreement with the Declaration of Helsinki.

Anthropometric Measurements

Weight and height were measured with subjects wearing light clothing and shoes removed. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m²). Subcutaneous fat thickness was measured using a skinfold caliper (Holtain® LTD, Crymych, UK) by the same trained technician at

three pre-defined locations (triceps, abdominal/suprailiac and thigh). The assessment of the body composition by using skinfold caliper is proven to be consistent with bioelectric impedance analysis [14].

Body density was estimated from the equation of Jackson and Pollack and the Siri's Formula was used to estimate the percentage body fat from body density [15, 16]. Fat-free mass (FFM) was determined as the difference between body mass and fat mass, where fat mass equaled body mass times percent body fat. FFM was corrected by the normalization method (FFM_{corr}) described in details by Ravussin and Bogardus [17].

Experimental Procedures

Subjects were instructed not to consume any food or beverages except water for 12 h before the test and not to engage in any type of physical exercise on the test day [18]. HRV and REE measurements were performed simultaneously between 8:00 and 09:30 a.m. in a semidarkened and quiet laboratory room specially prepared for REE measurements. Temperature and relative humidity were set to 20–24 °C and 50%, respectively by using an air-conditioning system.

Subjects rested for 10 min in a comfortable supine position with spontaneous breathing before the initiation of HRV and REE measurements. Then, HRV and REE were determined simultaneously while the subject lay face up for 20 min.

As it is known, breathing frequencies modify the influence of autonomic system on sinoatrial node [7]. In order to detect the effect of wearing a mask for metabolic measurements on the breathing frequencies of the participants, we recorded the breathing frequencies of the subjects for 1 min by visual observation at the 5th and 10th min of resting period, and it was compared with the breathing frequencies taken at the 10th and 20th min of REE measurements while subjects wearing a mask.

Metabolic Measurements

The REE measurement was assessed via the indirect calorimetry method (Vmax Spectra® 29c, Yorba Linda, CA, USA). The gas analyzer was calibrated prior to each test to certified calibration gases (26% O₂ and 74% N₂, 16% O₂ and 4% CO₂) according to the manufacturer's recommendations. Using a 3L syringe, the mass flow sensor was also calibrated before each experimental trial. The gas samples were collected breath by breath for 20 min on the supine position. The REE recordings were visually inspected, and the smoothest 5-min interval data in steady state condition were chosen to be used in the calculation of REE using the Abbreviated Weir Formula [19]. HRV was also obtained from the same time interval. All subjects were instructed not to move, talk or sleep during the REE measurement. The subjects were warned verbally in case of falling into sleep. Energy expenditure was determined during the follicular phase for all women to discard the influence of hormonal changes in the menstrual cycle during the measurement of REE [20]. REE (kcal/day) and REE which was adjusted for FFM (kcal/day/kg) were analyzed to assess the differences in metabolic rate between groups.

HRV Measurements

RR intervals were measured using the Polar Heart Rate Monitor (S810, Polar® Electro Oy, Kempele, Finland) for 20 min in supine position at a sampling frequency of 1000 Hz. The validity of the Polar S810 has been evidenced by studies of Gamelin et al. [21], Vanderlei et al. [22], and Nunan et al. [23]. The Polar S810 wrist receiver and chest strap were placed according to the manufacturer's recommendations.

HRV Analysis

HRV recordings derived from the POLAR S810 were transferred to a personal computer using the Polar Precision Performance 4.03 software. The raw HRV data were edited by the Polar Precision software (moderate filter power, minimum protection zone 6 bpm). The HRV record was discarded when the amount of abnormal beats was more than 2% [24]. Following artifact correction, RR intervals were exported under American Standard Code for Information Interchange (ASCII) format [21] to be analyzed with Kubois® HRV analysis Software v2 (Biosignal Laboratory, University of Kuopio, Kuopio, Finland) [25]. HRV analyses were performed in accordance with The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommendations [6]. A segment of 256 s was selected within the 300 s of the supine recording corresponding to the time of 5 min smoothest REE data to compute the mean RR interval [21]. The same segment used in the time domain analysis was resampled at 4 Hz and detrended for subsequent analysis. An autoregressive model was used to determine the power spectrum densities of the HRV analysis. The power spectrum for the frequency domain HRV analysis was

Table 1. Demographic anthropometric data of the groups^a

Variables	Group 1 (n = 18)	Group 2 (n = 20)
Age, years	31.06 ± 7.73	27.60 ± 6.36
Height, cm	171.67 ± 12.14	167.20 ± 8.49
Weight, kg	87.28 ± 17.37*	62.70 ± 8.05
BMI, kg/m ²	29.47 ± 3.87*	22.36 ± 1.54
Body fat, %	29.02 ± 6.94*	19.84 ± 7.26
Fat mass, kg	25.46 ± 9.45*	12.32 ± 4.68
FFMcorr, kg	74.55 ± 12.50*	63.12 ± 8.62

FFMcorr = Corrected fat-free mass.

^aData are means ± SD.

*Significantly different from group 2 (p < 0.05).

divided into three bands: very low frequency (VLF 0.0033–0.04 Hz), LF (0.04–0.15 Hz) and HF (0.15–0.40 Hz). Measurements of LF and HF power components expressed in normalized units (LFnu, HFnu), LF/HF ratio, and total power were assessed for statistical analysis [6].

Statistical Analysis

All statistical analyses were performed using SPSS for Windows v11.5.1 (SPSS® Inc. Chicago, IL, USA). The Shapiro-Wilk normality test was used to assess the normal distribution of the continuous variables separately for each group. According to normality test, all demographic and anthropometric data, except weight, were normally distributed in both groups. A log transformation was applied to total power and LF/HF that was not distributed normally. The Mann-Whitney nonparametric t test was used to assess the difference between groups in REE and FFMcorr. The independent t test was used to evaluate significant differences between groups for normally distributed variables. Chi-square test was used to assess gender distribution between groups. The relationship between REE and FFMcorr was assessed by linear regression test. Graph was drawn by use of GraphPad Prism Ver. 3.0 (GraphPad® Software Inc., San Diego, CA, USA).

Results

The subjects were divided into two groups according to their BMI. In group 1, there were 11 men and 7 women (10 subjects with BMI between 25.1 and 29.9 kg/m², 8 subjects with BMI ≥ 30 kg/m²). Group 2 consisted of 11 men and 9 women (BMI between 20 and 25 kg/m²). There were no significant differences between the groups in age and height. The gender distribution was not statistically different between groups ($\chi^2 = 0.145$, p = 0.703). Demographic and anthropometric characteristics of both groups were shown in table 1.

The mean breathing frequencies of 38 subjects were 16.63 ± 2.43 breaths/min in the resting time and 15.83 ± 3.75 breaths/min during the metabolic measurements. No significant difference was found between the resting time breathing frequencies and breathing frequencies in the metabolic measurement time in all participants (p > 0.05).

The mean RR was 911.79 ± 88.60 ms in group 1 and 908.18 ± 134.14 ms in group 2. There was no statistically difference between these two groups (p > 0.05). After log10 transformation was applied, the difference in total power between overweight/obese (2,776.17 ± 1,534.30 ms²) and normal-weight subjects (3,002.60 ± 2,562.86 ms²) was not significantly different (p > 0.05).

Fig. 1. The intercepts of the regression lines between REE and FFM.

The mean LFnu was 61.11 ± 17.09 in group 1 and 44.51 ± 18.63 in group 2. These values were significantly different ($p < 0.01$). The mean HFnu was 38.89 ± 17.09 in group 1 and 55.50 ± 18.63 in group 2, showing also a significant difference between the two groups ($p < 0.01$).

The LF/HF ratio after log10 transformation was 2.32 ± 2.09 in group 1 and 1.10 ± 1.05 in group 2 ($p < 0.01$).

Specifying REE data per kilogram, FFM is inappropriate to standardize the values of overweight/obese subjects and normal-weight subjects [17]. We calculated the intercepts of the regression line between REE and FFM for our study population by using multiple regression analysis. The regression formula was as follows: $REE = 274.37 + 21.74 \times FFM$, where the straight regression line, when extended, cuts the x-axis at the point $x = -12.74$; this value was used as a correction number for FFM (fig. 1). Since the total number of subjects (38 subjects) who participated in the study was low, we divided REE by FFM plus 12.74 kg (FFMcorr) [16]. The mean FFMcorr was 74.55 ± 12.50 kg in group 1 and 63.12 ± 8.62 kg in group 2, and there were statistically significant differences between the groups ($p < 0.05$) after adding correction number to FFM.

The mean REE was $1,597.04 \pm 327.83$ kcal/day in group 1 while it was $1,367.76 \pm 264.35$ kcal/day in group 2. The difference between two groups was statistically significant ($p < 0.05$). REE was adjusted for corrected FFM ($REE_{FFMcorr}$) according to the approach described above [16]. $REE_{FFMcorr}$ was 21.47 ± 2.92 kcal/day/kg in group 1 and 21.56 ± 1.90 kcal/day/kg in group 2; and this difference was not statistically significant ($p > 0.05$). It was found that in group 1 FFMcorr explained 52.8% and fat mass 4.2% of the variation in REE while the respective values in group 2 were 80.6% and 6.5%.

Discussion

Our study on spectral analysis of HRV demonstrated that although sympathovagal balance was elevated in overweight/obese subjects, REE adjusted for FFM was not different in overweight/obese and normal weight healthy subjects.

ANS has a vital role in the metabolic process related to the homeostatic regulation in the body. Cardiac autonomic activity can be assessed by spectral analysis of HRV. It was emphasized that the expression of LF and HF bands in normalized units reflects the relative

balance between sympathetic and parasympathetic control, and the LF/HF ratio can be used as an index of sympathovagal balance [7]. In the literature, the hypothesis of ‘an abnormality in ANS behavior in human obesity’ is the point that all the studies agreed with, but there is a lack of agreement concerning the SNS activity in obesity. Several studies suggested reduced activity of SNS [10] or depressions both in sympathetic and parasympathetic activity [26]. The changes in the ANS activity result in impairment in metabolic processes that can lead to weight gain and vice versa [5, 27]. Insulin and leptin are the hormones that can affect the CNS to reduce food intake by appetite regulation and to increase energy expenditure by stimulating the sympathetic system. Activation of the renin-angiotensin-aldosterone system is demonstrated in obesity before. Angiotensin can directly increase SNS activity [27]. It was reported that the balance between the sympathetic and parasympathetic nervous system can be altered in obese people [9]. The findings of our study showed that LFnu and LF/HF ratio increased and HFnu decreased in overweight/obese subjects compared to normal-weight subjects. These results may indicate an altered sympathovagal activity tending toward higher sympathetic activity and lower parasympathetic activity in overweight/obese subjects than in non-obese healthy subjects. In accordance with our hypothesis, Schmid et al. [9] and Lambert et al. [11] reported higher sympathetic activity in obese subjects.

REE in group 1 were statistically significantly higher than group 2. The differences in REE between groups can be attributed to higher FFM in overweight/obese subjects [28]. However, for the comparison of REE between obese and non-obese subjects, it is recommended to use REE per unit of metabolically active mass because there is nonlinearity between REE and FFM. When REE measurements were expressed per kg FFM it can be overestimated in subjects with low FFM or underestimated in subjects with high FFM [17]. To overcome this situation, multiple regression analysis was used to determine the metabolically active body mass of the two groups (FFM_{corr}). As a result of the analysis of REE adjusted for FFM_{corr} , we found that there was no difference between overweight/obese and normal-weight healthy subjects ($p > 0.05$). It was reported that REE decreases with weight loss and increases with weight gain [27, 29].

Elevated sympathovagal balance may lead to a higher REE level, but there was a close similarity of REE per kilogram of FFM_{corr} in overweight/obese and normal-weight healthy subjects in our study. Since our findings were determined by a cross-sectional study, there were no pre-obesity REE data of our subjects. REE might have been lower (like those in the normal-weight group) in group 1 in the pre-obesity period and gaining weight might have altered sympathovagal balance and induced REE to a new level. If the increment in REE in overweight/obese subjects had been higher than in non-obese subjects, it might have been thought that we could lose weight easily by dieting. Since $REE_{FFM_{corr}}$ was not high in overweight/obese subjects, additional methods such as exercise or calorie restriction are required to lose weight.

Based on the result of our study, FFM_{corr} explained 52.8% and fat mass 4.2% of the variation in REE while the respective values in group 2 were 80.6% and 6.5%. In accordance with our results, it was shown that in obese individuals the contribution of FFM to REE was greater than that of fat mass [30, 31]. The extracellular fluid (ECF) is also included in the FFM. Moreover, it was reported that adjusting FFM by ECF did not increase the ability of FFM to predict REE [30]. When the size of the metabolically active organ is increased, the active part of the tissue decreased; thus the metabolic activity of the larger FFM is lower than normal FFM [32]. Less metabolically active tissue may lead to lower contribution of FFM in the group 1.

One limitation of this study was that we were not able to analyze ANS activity by other methods, e.g., urinary catecholamine excretion and/or neural conductivity which may lead

to more accurate assessment of the SNS activity. Longitudinal studies are required to assess the REE and HRV interaction in the weight gaining process. In our study, the subjects in the overweight/obese group were rather young and did not suffer from any illness resulting from overweight. We accepted this period as a time where weight loss must begin to protect against the obesity-induced illness and death [33–35] and we advised the participants to join in a weight loss program in a hospital. It was also demonstrated that ANS activity and REE changed after weight loss [3, 29].

Conclusions

To avoid misestimation of the REE in overweight/obese and normal-weight subjects, the REE should be adjusted for corrected FFM. Contrary to our expectations, the findings of the present study indicated that $REE_{FFM_{corr}}$ levels in overweight/obese and non-obese subjects were not different despite elevated sympathovagal balance in overweight/obese individuals.

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Disclosure Statement

No conflict of interest was declared by the authors.

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