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Does circulating leptin play a role in energy expenditure?

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ABSTRACT

Objectives: It has been demonstrated that leptin influences the energy balance by regulating appetite and increasing energy expenditure (EE). However, the relationship between circulating leptin and EE is confounded owing to variations in body composition. The aim of this study was to determine the role of circulating leptin in energy regulation and to examine whether the leptin-mediated changes in EE are associated with adiposity among healthy adults living in Singapore.
Methods: We conducted a cross-sectional study of 300 participants (112 men). Resting metabolic rate (RMR) was measured by indirect calorimetry. Body composition (i.e., fat mass [FM] and fat-free mass [FFM]) was measured by dual-energy x-ray absorptiometry. Serum leptin levels were determined by radioimmunoassay.
Results: There were strong correlations between circulating leptin levels, FM, and RMR in healthy men and women. After normalization of RMR by a power function model (kcal·kg $FFM^{0.86}$·d^{-1}), the influence of FFM can be effectively removed. The normalized RMR was significantly associated with both FM ($r = 0.28$, $P < 0.001$) and log leptin ($r = 0.35$, $P < 0.001$). In the stepwise multiple regression analysis, leptin level is the major predictor for normalized RMR, accounting for 12% of the variation. In contrast, FM did not explain any variation in normalized RMR.
Conclusion: Leptin may be a more significant predictor of normalized RMR than FM per se. The contribution of FM to RMR could be via a mechanism that is related to leptin-dependent responses involved in energy homeostasis.

Introduction

The analysis and interpretation of energy balance (EB) is at the heart of obesity research. The global obesity epidemic and its burden on social and economic costs, heighten the necessity for new insights into mechanisms that govern EB [1]. EB is made up of energy intake (EI) and energy expenditure (EE), that is, EB = EI − EE. Thus, numerous studies have been conducted to understand the mechanisms that regulate both EI and EE. Leptin, an adipocyte-derived hormone, has been demonstrated to regulate food intake and EE [2,3]. As a peripheral signal that informs the brain of the metabolic state, it is believed that leptin increases EE through its effects on the cardiovascular system and brown adipose tissue (BAT) thermogenesis via the hypothalamus [4].

Although leptin has shown a clear-cut effect on metabolic rate in ob/ob mice [5–7], its applicability to energy homeostasis in humans remains contentious. Some studies reported positive correlations between leptin concentrations and EE in healthy men [8] as well as in elderly African American women [9]. However, other studies found no such associations [10–12]. The inconsistencies in these results may be due to methodological factors. When investigating the relationship between EE and leptin, cautious consideration should be given to the body composition of the individuals studied. The human body can be divided into fat mass (FM) and fat-free mass (FFM). FM is not only related to circulating leptin levels [13,14] but more importantly, both FM and FFM are predictors of resting metabolic rate (RMR). RMR represents ≤70% of total EE [15]. The influence of body composition variations per se must be first statistically eliminated from RMR before analyzing the relationship between leptin and EE [16,17].

In light of the conflicting results on the relationship between leptin and EE and the availability of limited data points obtained in Asian populations, the aim of the present study was to determine the role of circulating leptin in energy regulation and to examine whether the leptin-mediated changes in EE are associated with adiposity among healthy adults living in Singapore, a country in southeast Asia.

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Methods

Study design

This study is based on a cross-sectional sampling design to collect health-related information from 300 healthy Chinese adults 21 to 74 y of age (112 men [37.3%] and 188 women [62.7%]). Participants were recruited through advertisements in local newspapers and posters that were placed around the National University of Singapore campus, public areas, and on the Clinical Nutrition Research Centre (CNRC) website. To be eligible, participants were required to be Singaporeans or permanent residents who have resided in Singapore for ≥5 y and to be healthy. Participants were excluded if they were diagnosed with any major diseases or took regular medication. Women who were pregnant or used hormonal contraceptives were excluded. Before the study, all participants were asked to restrict alcohol- and caffeine-containing drinks and to refrain from intense physical activity within 24 h. All procedures were approved by the National Healthcare Group Domain Specific Review Board, Singapore.

Anthropometry

Participants arrived at the Clinical Nutrition Research Centre laboratory in the morning after a 10-h overnight fast. All participants gave written informed consent before starting. Weight (kg) in light clothing without footwear was measured to the nearest 0.1 kg and height (cm) was measured to the nearest mm using a digital scale (Seca 763, Birmingham, United Kingdom). Body mass index (BMI; kg/m^2) was calculated using weight divided by the height squared. The waist circumference (WC; cm) was measured with an ergonomic circumference measuring tape above the umbilicus or navel and below the xiphoid process. All the measurements were done in duplicate and readings were averaged.

Dual-energy x-ray absorptiometry (DXA; QDR 4500 A, fan-beam densitometer, Hologic, Waltham, MA, USA) was used for the measurement of FM (kg) and FFM (kg). FM (%) was calculated by using the manufacturer's software (software version 8.21).

Serum leptin levels and energy expenditure

We collected 10 mL of venous blood in Vacutainers (Becton Dickinson Diagnostics, Franklin Lakes, NJ, USA). Blood samples were separated by centrifugation at 1500*g* for 10 min at 4°C within 2 h of being drawn and aliquots were stored at −80°C until analysis. Fasting serum leptin levels (ng/mL) was measured using radioimmunoassay as described elsewhere [18].

RMR was measured using an indirect calorimeter (COSMED, Rome, Italy) in the fasted state. Participants were instructed to lie in a supine position for 30 min, in a room maintained at thermo-neutral temperature (22°C–26°C), without moving while a plastic canopy with veil was placed over the upper part of their bodies to prevent loss of expired air [19]. During the period of measurement, participants were instructed to remain awake and to limit movement. RMR was measured using the last 10 min of the measurement period to ensure stable and interpretable measurements were obtained [20].

Statistical analysis

Statistical analysis was performed using the SPSS version 23 (IBM, Armonk, NY, USA). All data are expressed as means ± SD. One-way analysis of variance was used for between-group comparisons. The strength of the associations between leptin levels and clinical variables were assessed by partial correlations after adjustment for the potential confounders. Stepwise linear regression models were fit for leptin or normalized RMR as a dependent variable, including FFM, FM, age, and sex as independent variables to demonstrate the relative contribution of each of these variables to the outcome variable. Two-sided $P < 0.05$ was considered statistically significant in all cases.

To normalize RMR, we used a power function model to identify appropriate normalizing parameters:

$$RMR = \alpha \cdot X_1^{\beta 1} \cdot X_2^{\beta 2} \cdots X_k^{\beta k} \cdot \varepsilon$$

Where X_1, X_2, ...X_k are the predictors (e.g., FFM, FM, and age) α, β_1, . . . β_k are unknown parameters that have to be estimated, and ε is a multiplicative error term. We carried out model validation using residual analysis and goodness-of-fit tests. Data splitting was used for cross-validation.

Results

Table 1 shows the baseline characteristics of the study population. Women had significantly higher leptin levels and FM than men. In contrast, men had significantly higher BMI, WC, FFM, and RMR than women. The association of leptin levels with various anthropometric characteristics, body composition, and RMR are presented in Table 2. After adjustment for age, smoking status, supplementary usage, family disease history, physical activity, and menopause status (only in women), circulating leptin levels showed significant correlations with all body composition variables and RMR in both men and women. The strongest correlation was observed between leptin and FM (kg) for both sexes.

Table 1
Characteristics of the study population*

Variables	Total (N = 300)	Men (n = 112)	Women (n = 188)	*P*-value†
Age (y)	43.3 ± 13.7	43.6 ± 14.1	43.2 ± 13.5	0.794
Height (cm)	163.5 ± 8.3	171.1 ± 6.2	159 ± 5.6	<0.001
Weight (kg)‡	59.9 ± 11.7	67.8 ± 9.5	55 ± 10.2	<0.001
BMI (kg/m^2)	22.5 ± 3.3	23.5 ± 2.8	21.9 ± 3.5	<0.001
WC (cm)	74.6 ± 9.2	80.5 ± 7.7	71.1 ± 8.2	<0.001
FM (%)‡	31.8 ± 7.3	25.6 ± 5.2	35.5 ± 5.7	<0.001
FM (kg)‡	18.9 ± 6.1	17.6 ± 5.4	19.7 ± 6.3	0.005
FFM (kg)‡	40.6 ± 9.1	50.2 ± 5.9	34.8 ± 4.8	<0.001
Leptin (ng/mL)	11.8 ± 11.3	5.5 ± 4	15.3 ± 12.5	<0.001
RMR (kcal/d)	1240 ± 233	1434 ± 192	1122 ± 166	<0.001

BMI, body mass index; WC, waist circumference; FM, fat mass; FFM, fat-free mass; RMR, resting metabolic rate.
*Values expressed as mean ± SD.
†One-way analysis of variance.
‡Measured by dual-energy x-ray absorptiometry.

Table 3 shows the stepwise linear regression using leptin as a dependent variable, FFM, FM, age, and sex as independent variables. It was found that FFM was a dominant predictor of RMR, explaining 74% of the variation in RMR. FM and age made minor contributions (4% and 2%, respectively) to the variation in RMR. To remove the influence of body composition and age on RMR, we used a power function model to identify appropriate normalizing exponents for RMR. The exponent for the normalizing RMR by FFM was found to be 0.86 by multiple linear regression with log RMR as the dependent variable and log-FFM, log-FM, and log-age as the independent variables. Because of the minor contributions to the variance, both FM and age were excluded from the model. Figure 1 shows that there was no association between the allometrically normalized variable RMR (i.e., RMR/FFM$^{0.86}$) and FFM ($r = 0.02$, $P = 0.854$ for men, $r = -0.03$, $P = 0.728$ for women).

We also investigated whether the contribution of FM to normalized RMR was leptin dependent. Figure 2 shows that normalized RMR was significantly associated with FM ($r = 0.28$, $P < 0.001$) and log-leptin ($r = 0.35$, $P < 0.001$). Stepwise multiple linear regression was fit for normalized RMR as a dependent variable, including FM, log-leptin, age, and sex as independent variables (Table 4). Table 4 shows that leptin level was a dominant predictor of the normalized RMR, explaining 12% of the variation. Age and sex were minor

Table 2
Partial correlation coefficients between body composition, RMR, and leptin after adjustment for age, smoking status, supplementary usage, family disease history, physical activity, and menopause status (only in women)

Variables	Men (n = 112)	Women (n = 188)
Weight (kg)	0.59*	0.66*
WC (cm)	0.71*	0.71*
BMI (kg/m^2)	0.66*	0.76*
FM (%)	0.80*	0.76*
FM (kg)	0.82*	0.80*
FFM (kg)	0.25†	0.33*
RMR (kcal/d)	0.31*	0.42*

BMI, body mass index; FFM, fat-free mass; FM, fat mass; RMR, resting metabolic rate; WC, waste circumference.
*$P < 0.005$.
†$P < 0.05$.

Table 3
Stepwise multiple linear regression analysis with RMR as the dependent variable

Independent variables	Regression coefficients (SE)	95% CI	*P*-value	Adjusted R^2 (%)
FFM (kg)	0.021 (0.001)	0.019 to 0.022	<0.001	73.9
FM (kg)	0.008 (0.001)	0.006 to 0.010	<0.001	3.5
Age (y)	−2.518 (0.462)	−3.428 to −1.608	<0.001	2.1

FFM, fat-free mass; FM, fat mass; RMR, resting metabolic rate.

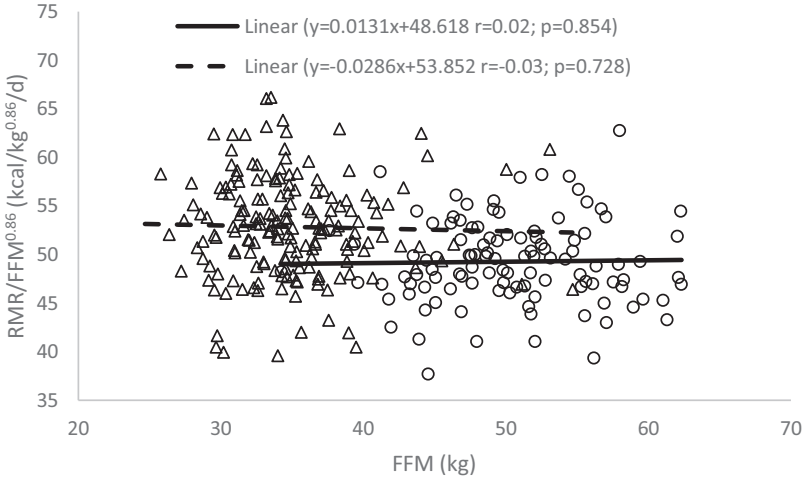

Fig. 1. Normalizing RMR as a power function model (kcal·kg $FFM^{0.86}$·d^{-1}) removes the influence of FFM on RMR. Men are represented by open circles and solid line and women by open triangles and dashed line. FM, fat mass; FFM, fat-free mass; RMR, resting metabolic rate.

determinants (explaining 4% and 2%, respectively) of the variation of the normalized RMR. FM did not explain variation in normalized RMR. The results in Figure 2 and Table 4 suggest that normalized RMR was more determined by circulating leptin level than by FM.

Discussion

Owing to the significant association between EE and body composition, appropriate normalization of EE to body composition is critical [21]. However, the optimal or ideal method for EE normalization in human is still under debate. As shown here and elsewhere [22–24], simple ratio-based normalization methods whereby RMR is divided by body weight or FFM do not adequately account for the effect of variation in body mass (Supplementary Fig. 2). By comparison, the use of curvilinear power function model is physiologically superior to simple linear models [25–27]. Herein, we employ a power function model to normalize RMR and address whether the normalized RMR can help to clarify the link among body composition, EE, and circulating leptin. Combined with our findings that FFM is a superior but FM is a minor predictor of RMR, our results support the use of power function model (i.e., kcal·kg $FFM^{0.86}$·d^{-1}) to normalize RMR. The influence of FFM variation on RMR is effectively controlled with this model.

Previous studies suggest that FM is an independent contributor to RMR [28–31]. The link between FM and RMR is possibly due to the compensatory responses involved in energy homeostasis triggered by changes of adipose tissue [31]. The individual with high body fatness normally has an adaptive increase of RMR that depends on the sympathetic nervous system (SNS) and acts to limit weight gain [32]. The major contributor to the adaptive thermogenesis in small mammals is brown adipose tissue (BAT) [33]. However, the role of BAT in thermogenesis in adults remains unclear. In humans, skeletal muscle work efficiency is modulated in response to changes of EE [34]. Both BAT thermogenesis and efficiency of skeletal muscle work may contribute to the mechanism linking FM and EE.

Hormone leptin is secreted from adipose tissue and is critical to inform the central nervous system about the status of peripheral energy reserves [3,35]. Since its discovery, leptin has been studied regarding its ability to activate SNS-mediated changes of BAT thermogenesis and skeletal muscle work efficiency [34]. The role of leptin signals on three different hypothalamic nuclei (i.e., the dorsomedial hypothalamus, the ventromedial hypothalamus, and the arcuate nucleus) [4] to integrate afferent signals pertinent to EB. Therefore, the elevated RMR in an individual with high body fatness could result, at least in part, from higher leptin levels. However, the causal relationship among RMR, FM, and leptin will need to be examined in both prospective and interventional studies. Nonetheless, our observation that FM was positively associated with normalized RMR (eliminates the influence of FFM) confirmed that FM is an independent contributor to RMR. Moreover, the contribution of FM to variation of normalized RMR disappeared in the stepwise multiple regression analysis. Circulating leptin was a predominant predictor for normalized RMR variation. These results, when combined, allowed us to conclude that the contribution of FM on RMR may be leptin dependent. Hence, circulating leptin may play a more direct role to determine RMR than FM per se.

This study had several limitations. First, because of its cross-sectional design, it was not possible to explore the causal relationship among EE, circulating leptin, and body composition. Second, although we evaluated a relatively large number of participants, the participants in this study were generally lean (mean BMI 22.5 kg/m^2) and healthy. Because obesity may promote multiple cellular processes that attenuate leptin signaling [36], the association between leptin and EE in obese patients may need to be further examined in both prospective and interventional studies.

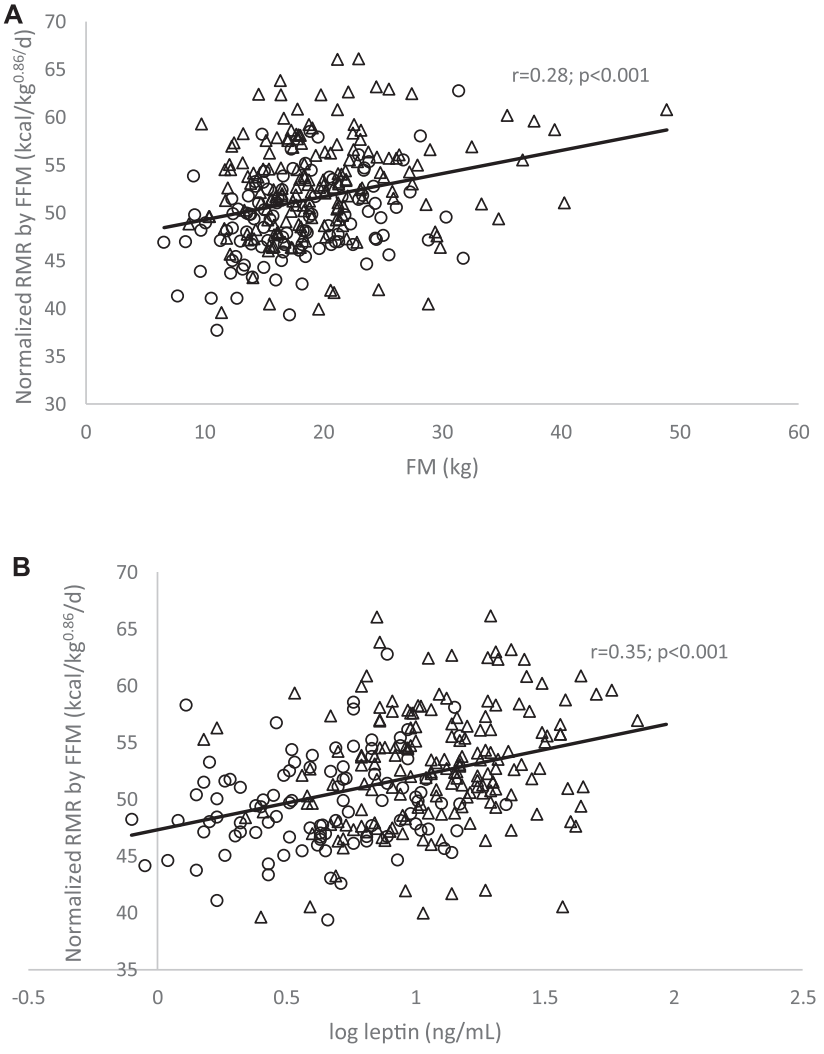

Fig. 2. Associations of normalized RMR with (**A**) FM ($r = 0.28$, $P < 0.001$) and (**B**) log-leptin ($r = 0.35$, $P < 0.001$). Men are represented by open circles and women by open triangles. FM, fat mass; RMR, resting metabolic rate.

Table 4
Stepwise multiple linear regression analysis with normalized RMR as the dependent variable

Independent variables	Regression coefficients (SE)	95% CI	*P*-value	Adjusted R^2 (%)
Log leptin (ng/mL)	3.625 (0.892)	1.868 to 5.381	<0.001	12.3
Age (y)	−0.073 (0.020)	−0.113 to −0.034	<0.001	4.2
Sex	1.599 (0.694)	0.234 to 2.965	0.022	1.6

RMR, resting metabolic rate.

Conclusions

Results from the present study demonstrated that body composition (i.e., FFM and FM) acts as a confounding factor when investigating the relationship between circulating leptin levels and RMR. Normalizing RMR by using a power function model can effectively remove the effect of body composition variation. FM contributes to RMR in the present participants via mechanisms that are related to leptin-dependent adaptive responses involved in the energy homeostasis.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.nut.2018.08.015.

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