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Modelling the associations between fat-free mass, resting metabolic rate and energy intake in the context of total energy balance

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The authors declare no conflict of interest.

ABSTRACT

Background: The relationship between body composition, energy expenditure and ad libitum energy intake has rarely been examined under conditions that allow any interplay between these variables to be disclosed.

Objective: The present study examined the relationships between body composition, energy expenditure and energy intake under controlled laboratory conditions in which the energy density and macronutrient content of the diet varied freely as a function of food choice.

Methods: Fifty nine subjects (30 men: mean body mass index = 26.7 ± 4.0 kg/m^2; 29 women: mean body mass index = 25.4 ± 3.5 kg/m^2) completed a 14 day stay in a residential feeding behaviour suite. During days 1 and 2, subjects consumed a fixed diet designed to maintain energy balance. On days 3-14, food intake was covertly measured in subjects who had ad libitum access to a wide variety of foods typical of their normal diets. Resting metabolic rate (respiratory exchange), total daily energy expenditure (doubly labelled water) and body composition (total body water estimated from deuterium dilution) were measured on days 3-14.

Results: Hierarchical multiple regression indicated that after controlling for age and sex, both fat-free mass ($p < 0.001$) and resting metabolic rate ($p < 0.001$) predicted daily energy intake. However, a mediation model using path analysis indicated that the effect of fat-free mass (and fat mass) on energy intake was fully mediated by resting metabolic rate ($p < 0.001$).

Conclusions: These data indicate that resting metabolic rate is a strong determinant of energy intake under controlled laboratory conditions where food choice is allowed to freely vary and subjects are close to energy balance. Therefore, the conventional adipocentric model of appetite control should be revised to reflect the influence of resting metabolic rate.

INTRODUCTION

Over the last 60 years there has been great interest in physiological signals that regulate appetite and energy balance (1). Numerous models predict that certain components of energy and nutrient balance act as negative feedback signals in appetite and body weight control (1). Specific aspects of nutrient balance such as carbohydrate oxidation (2) or stores (3), fat stores (4) or body weight per se (5) have been proposed as key peripheral signals that exert negative feedback on energy intake (EI). The discovery of leptin (6) appeared to provide a molecular basis for Kennedy's 'lipostasis' concept (4, 7), and stimulated intense focus on adipose derived signals in energy balance regulation. However, while the importance of leptin should not be underplayed, secular trends in obesity prevalence (8, 9) indicate that adipose tissue accumulation does not exert strong negative feedback to restore energy balance, at least from the point of excess EI. Indeed, despite this focus on leptin and other adipose derived feedback signals (5, 10, 11), there is remarkably little evidence in humans on the extent to which changes in adipose tissue exert feedback on EI at the whole body level.

Evidence in humans suggests that the metabolism or storage of specific macronutrients fails to exert powerful negative feedback on EI (1, 12, 13). However, models that include all macronutrients explain greater variance in EI. Therefore it is important to examine how changes in nutrient stores and metabolism collectively influence EI. Despite the critical role of protein-energy relationships for survival time during under nutrition (14-16), few have analysed energy expenditure (or its determinants) as major sources of feedback in appetite control (17, 18). Therefore, while intuitive to speculate that EI is driven by energy needs, it has not been convincingly demonstrated that energy expenditure influences the control of ad libitum energy intake.

Recently it has been shown that fat-free mass (FFM), but not fat mass (FM), predicts ad libitum meal size and daily EI in overweight and obese individuals (19). These findings are in agreement with earlier observations (20, 21) and have been independently replicated (22). Therefore, it has been proposed that FFM, as the main determinant of resting metabolic rate-(RMR), drives EI at a level proportional to basal energy requirements (23). In support of this, RMR was found to predict hunger and objectively measured EI in overweight and obese subjects (24). However, these findings need to be confirmed in the context of total energy balance, particularly as FFM and RMR co-vary strongly and little is known about their individual contributions to EI. Therefore, the present study aimed to examine the relationships between body composition, energy expenditure and ad libitum EI under controlled laboratory conditions in which food choice was allowed to vary freely.

SUBJECTS AND METHODS

Subjects

Fifty nine volunteers (30 men and 29 women) were recruited from the Aberdeen area (Table 1). Subjects were stratified into three age categories (20-35 years, 36-50 years and 51-65 years) and two BMI categories (BMI 20-25 kg/m^2 and BMI >25 kg/m^2). Subjects were non-smokers, free from disease and not taking medication known to effect metabolism or appetite. Menopausal and physical activity status were not included as part of this exclusion criteria. Prior to the start of the study written informed consent was obtained and ethical approval was granted by the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen. Subjects were informed that the purpose of the study was to examine the relationships between diet and lifestyle.

Table 1 here

Study Design

Daily energy and macronutrient intake was objectively measured during a 14-day residential stay in the Human Nutrition Unit (HNU) at the Rowett Institute of Nutrition and Health. During days 1-2, subjects consumed a fixed diet designed to maintain energy balance, with EI estimated at 1.5 and 1.6 times RMR for women and men, respectively. The proportion of energy contributed by fat, protein and carbohydrate to daily EI was 35%, 15% and 55%, respectively. During days 3-14, food intake was covertly measured in subjects who had ad libitum access to a wide variety of foods typical of their normal diets. Resting metabolic rate (respiratory exchange) and body composition (total body water estimated from deuterium dilution) was measured on day 3, while total daily energy expenditure (doubly labelled water) was measured over days 3-14. During their residence subjects were asked to maintain their normal behaviour as much as possible. Subjects were able to move freely around the HNU and associated grounds (under supervision of a member of staff), and had access to an exercise bike and treadmill during their stay. Subjects were also free to leave the HNU during the study, but were accompanied and observed by a member of staff at all times. The current analysis is based on a previous study examining the accuracy of food intake reporting (25), which had no a priori hypotheses about the relationship between physiological and behavioural measurements.

Procedures

Resting Metabolic Rate

Resting metabolic rate was measured by indirect calorimetry over 30–40 minutes using a ventilated hood system (Deltatrac II, MBM-200, Datex Instrumentarium Corporation, Finland). Subjects laid on a bed in a thermo-neutral room and were instructed to lie still but not to fall asleep. Resting energy expenditure was calculated from minute-by-minute data using the mean of 15 minutes of stable measurements, with the first and last 5 minutes

excluded. The equations of Elia and Livesey (26) were used to derive RMR. Details of calibration burns and repeatability testing have been described previously (27).

Body Composition

Stature was measured to the nearest 0.5 cm on day 3 of the study using a portable stadiometer. Body mass was measured to the nearest 0.01 kg on days 3-14 after voiding using calibrated digital scales (DIGI DS-410; CMS Weighting Equipment). Total body water was measured by deuterium dilution (see below) as described by Pullicino, Coward, Stubbs & Elia (28) and Coward (29). Fat-free mass was then subsequently estimated by assuming a hydration factor of 0.73 and that total body fat is hydrophobic. Fat mass was estimated as body mass minus FFM.

Total Daily Energy Expenditure

Total daily energy expenditure was measured on days 3-14 using doubly labelled water. On the morning of day 3, subjects were woken (07.00 hours), emptied their bladders and weighed. At 09.00 hours, subjects provided a baseline urine sample, which was used alongside two background samples collected during days 1 and 2 to provide information on the pre-dose isotopic enrichment of the subjects' body water pools. Immediately after the 09.00 hour sample subjects consumed orally a pre-prepared dose of $^{2}H_{2}{}^{18}O$, and100 ml of tap water to prevent the isotope being lost from the buccal cavity. The dose levels were 0·15 g/kg body mass of a 99% $^{2}H_{2}O$–$H_{2}O$ mixture and 1·5 g/kg body mass of a 10·0% $H_{2}{}^{18}O$–$H_{2}O$ mixture for subjects one to 42 and 44. Dose levels of oxygen 18 were reduced to 0·9 g/kg body mass for the remaining nineteen subjects because of the world shortage in doubly labelled water at the time of the experiment. Following this dose, subjects collected urine samples at 4, 5 and 6 hours post administration to enable the plateau to be individually measured.

On days 4-14, subjects provided urine samples at 11.00 hours under supervision and these were frozen (-20°C) until analysis. To calculate energy expenditure, urine samples were used for a multi-point stable-isotope analysis using gas isotope ratio MS. The log transformed data of enrichment by time were extrapolated back to time 0, giving a theoretical enrichment at time 0, which provided information on the individual's size of the body water pool assuming the dilution principle. Isotopic enrichment of the post-dose urine samples was analysed relative to the original background amounts. Pool sizes and flux rates were calculated as described by Coward (29). Energy expenditure was calculated from CO_2 production using the Weir equation (30):

- $EE = 4{\cdot}63CO_2 + 16{\cdot}49(CO_2/\text{ respiratory quotient})$,

The food quotient was substituted for respiratory quotient as it was assumed to be equivalent (31). The food quotient was calculated from macronutrient intakes taken from the laboratory weighed intakes after adjusting for changes in fat stores resulting from energy imbalance over days 3-14, and assuming an energy value of 29 MJ/kg and that all changes in body stores were in the form of fat (31). This energy cost was for the purposes of estimating the respiratory quotient in calculation of energy expenditure from doubly labelled water only and not for estimating the cost of weight gain or loss.

Energy and Macronutrient Intake

On days 3-14, food intake was covertly and objectively measured in subjects who had ad libitum access to a wide variety of foods from their normal diet. Food intake was measured overtly by subjects for two, 3-day periods during days 3-14 (with the order randomized). Based on 7-day diet histories and shopping list records collected prior to the start of the study, an inventory of foods and beverages typically consumed by each subject in their normal diet was purchased. If subjects reported an item usually consumed in their habitual diet was missing, this was subsequently purchased and made available.

147

148 During days 3-14, each subject had access to their own individual kitchen, which consisted of
149 a fridge, freezer and a cupboard containing their pre-selected foods and beverages. Subjects
150 only had access to their own kitchen. Subjects were able to freely select what and when they
151 wanted to eat (based on their own foods and beverage items), and meals were cooked by
152 subjects in their own kitchens. Subjects were instructed to leave all food waste, peelings and
153 packaging in special bins in their kitchens. Dishes/cooking utensils used were placed in a
154 specific section of their kitchen and subjects were instructed not to wash these.

155

156 Each morning a researcher entered the kitchens before the subjects woke and re-weighed all
157 the food items and any left-overs, peelings and packaging to the nearest 0.1 g (Soehnle model
158 820; Soehnle-Waagen GmbH or Ravencourt model 333; Ravencourt). These weighed intakes
159 were used to calculated 24 hour EI, with energy and nutrient content calculated using dietary
160 analysis software (Diet 5, Robert Gorden University, Aberdeen).

161

162 **Statistical Analysis**

163 Data are reported as mean ± SD unless otherwise stated. Statistical analyses were performed
164 using IBM SPSS for windows (Chicago, Illinois, Version 21). A paired t-test was used to
165 examine for differences between mean daily EI and mean daily energy expenditure.
166 Furthermore, a Bland and Altman plot was used to compare the deviations between the
167 methods used for the assessment of energy balance. To examine the relationships between
168 body composition, energy expenditure and daily EI, hierarchical multiple regression was
169 used. Three separate models were tested for the prediction of EI. In model 1, RMR was
170 examined after adjusting for energy density. In model two, RMR was tested as an
171 independent predictor of EI after FFM and FM were included. In model three, RMR was

examined with total daily energy expenditure. Given their known effect on EI, sex and age were included as covariates in all models.

A path analysis was conducted to further examine the associations between FFM, FM, RMR and EI. A model was tested examining whether the associations between body composition (FFM and FM – independent, exogenous variables) and EI (dependent, endogenous variable) would be mediated by RMR (endogenous mediator variable). The significance of the regression coefficients and fit statistics were calculated using the Maximum Likelihood estimation method. The following recommended goodness of fit indices were analysed to test for the adequacy of the mediation model: Chi-square (χ^2), Tucker Lewis Index (TLI), Comparative Fit Index (CFI), and Root-Mean Square Error of Approximation (RMSEA), with 95% confidence interval (32, 33).

The assumptions of uni and multivariate normality of errors were assessed by skewness and kurtosis coefficients. There was no severe violation of the normal distribution (33), with skewness values ranging from 0.35 (FM) to 1.07 (EI), and with kurtosis values ranging from 0.67 (FFM) to 2.49 (EI). The significance of the direct, indirect and total effects was assessed using Chi-Square tests (33). The Bootstrap resampling method was further used to test the significance of the mediational paths, using 2000 Bootstrap samples and 95% bias-corrected confidence intervals (CI) around the standardized estimates of the effects. Effects were regarded as significantly different from zero ($p < 0.05$) if zero was not included in the interval between the lower and the upper bound of the 95% bias-corrected CI (33). The software AMOS (Analysis of Momentary Structure, software version 18, SPSS Inc. Chicago, IL) was used to estimate the path analysis.

RESULTS

Validation of the Laboratory-Weighed Intakes

Table 2 here

Mean daily EI, energy expenditure, energy balance and the rate of body mass change can be seen in Table 2. In order to examine the validity of the laboratory weighed intakes, daily EI was compared to daily energy expenditure. This validation is based on the principle that:

- EI = total energy expenditure ± Δ body stores.

No significant differences existed between mean daily EI and the mean daily energy expenditure ($t = 0.731$, $df = 58$, $p = 0.468$). Furthermore, the relationship between EI and energy expenditure was expressed using a Bland-Altman plot in order to illustrate the spread of the differences (EI - energy expenditure) against the mean of the two methods. As can been seen in Figure 1, there was a good spread in the data and there were no systematic trends. Further details of the relationships between EI - energy expenditure and energy balance estimated from change in body mass are given in a previous publication and online supplementary materials (25). These data indicate that the procedures used in the present study provided a valid measure of daily ad libitum EI.

Predictors of Daily Energy Intake

In order to examine the relationships between body composition, energy expenditure and EI, three separate hierarchical multiple regression models were used (Table 3). In Model 1, energy density was added in the first step ($F_{(1, 57)} = 20.045$, $p < 0.001$), and accounted for 26.0% of the variance in daily EI. The addition of RMR (step 2) significantly improved the model ($F_{(2, 56)} = 45.140$, $p < 0.001$; $R^2 = 0.617$), accounting for a further 35.7% of unique variance in EI. During this final step, both energy density (ß = 0.390; $p < 0.001$) and RMR (ß = 0.610; $p < 0.001$) independently predicted EI (Figure 2).

In Model 2, step 1 accounted for 29.9% of the variance in daily EI ($F_{(2, 56)} = 11.947$, $p < 0.001$), with FFM (ß = 0.514; $p < 0.001$), but not FM (ß = 0.096; $p = 0.410$), independently predicting EI. Again, the addition of RMR further improved the model (Step 2; $F_{(3, 55)} = 16.769$, $p < 0.001$; $R^2 = 0.478$), accounting for an additional 17.9% of unique variance in EI. During this final step, only RMR independently predicted EI (ß = 0.675; $p < 0.001$).

Table 3 here

In Model 3, RMR was added in the first step and accounted for 47.4% of the variance in EI ($F_{(1, 57)} = 51.358$, $p < 0.001$). In step 2 ($F_{(2, 56)} = 28.661$, $p < 0.001$; $R^2 = 0.506$), the addition of total daily energy expenditure failed to further improve the model ($\Delta R^2 = 0.032$; $p = 0.063$), with RMR the only independent predictor of EI (ß = 0.536; $p < 0.001$). For each model, age, BMI and sex were also entered in a final Step. However, the addition of these variables failed to influence the reported outcomes, and therefore, these variables were not included for analysis in the reported models.

Figure 2 here

Path Analysis

The hypothesised model was tested through a fully saturated model that included 14 parameters. Results indicated that the paths regarding the direct effects of FM on EI ($b_{FM} = -0.018$; SEb = 0.034; Z = -0.529; $p = 0.597$; $\beta = -0.055$), and FFM on EI ($b_{FFM} = 0.013$; SEb = 0.041; Z = 0.331; $p = 0.740$; $\beta = 0.05$), exceeded the critical value for two-tailed statistical significance at the 0.05 level (Figure 3). These non-significant paths were removed and the model was recalculated.

Results showed that the adjusted model presented an excellent model fit, with a non-significant chi-square [$\chi^2_{(2)} = 0.415$ $p = 0.813$], and as supported by the other selected fit

indices: TLI = 1.053; CFI = 1.000; RMSEA = 0.000 (p = 0.835). All path coefficients were statistically significant ($p < 0.05$), and the model accounted for 47% of EI variance. Fat mass and FFM were significantly correlated and accounted for 61% of RMR, with a direct effect of 0.224 (b_{FM} = 32.942; SEb = 12.526; Z = 2.630; p = 0.009) and 0.691 (b_{FFM} = 88.123; SEb = 10.849; Z = 8.123; $p < 0.001$), respectively. Only RMR presented a significant direct effect (β = 0.688) on EI (b_{RMR} = 0.002; SEb = 0.000; Z = 7.229; $p < 0.001$).

Regarding the mediational tests, results indicated that FM presented an indirect effect of 0.154 on EI mediated by increased RMR. Also, FFM predicted increased EI with an indirect effect of 0.476, again through increased RMR. According to the Bootstrap resampling method, the estimates of the indirect effects of FM (CI = 0.045 to 0.278, p = 0.006) and FFM (CI = 0.312 to 0.610, p = 0.001) on EI, framed by a CI of 0.95%, were significantly different from zero.

Figure 3 here

DISCUSSION

This study examined the relationship between body composition, energy expenditure and EI in subjects at or close to energy balance under ad libitum feeding conditions. Resting metabolic rate was found to be a strong independent predictor of EI when the energy density and macronutrient composition of the diet varied freely as a function of food choice. These data suggest a fundamental (and robust) associations between RMR and the energy acquired through food, and add to previous research indicating that the energy needs of the body may well play an important role in day-to-day food intake (19-22, 24).

Some theories of appetite control embody the view that episodic and tonic inhibitory signals arising from adipose tissue and gastrointestinal peptides modulate a constant excitatory drive to eat (34). However, the source of this excitatory drive has been poorly defined, with current models of appetite control better able to account for the inhibition rather than initiation of feeding (35). Furthermore, such models do not incorporate energy expenditure as putative signals of food intake. Importantly, the present findings indicate that the energy expenditure arising from RMR stimulates food intake, and helps account for this excitatory drive. This tonic signal of energy demand would help 'tune' EI to energy expenditure and ensure the execution of key biological processes (23).

While lean tissue acts as an orexigenic feedback signal following semi-starvation (36, 37), there has been less attention on the role that skeletal or lean mass plays in day-to-day food intake. Previous studies have reported that FFM, the main determinant of RMR (38), predicts food intake in obese individuals (19, 20, 22). In agreement with these studies, FFM (but not FM) predicted daily EI in the present study. However, once RMR was included in the regression model, FFM failed to independently predict EI. As such, the effect of FFM on EI appeared to be mediated by, rather than independent of, RMR. These effects were confirmed by a mediation model using path analysis in which the effect of FM and FFM on EI was fully mediated by RMR. While path analysis is a robust statistical procedure that allows tests for hypothesized causal relationships to be conducted, caution must be taken when using relatively small samples (33). Nonetheless, the model complexity and data used followed required assumptions to conduct the analysis, and the estimation technique applied has been found to provide valid and stable results in simulation studies with samples with similar dimensions (32).

297 Resting metabolic rate has previously been shown to a determinant of ad libitum meal size
298 and daily EI (24), although food choice was restricted in this study. In contrast, subjects in
299 the present study had ad libitum access to a wide range of foods typical of their normal diet,
300 and dietary energy density and macronutrient composition varied as a function of food choice.
301 This is of importance as energy density is a potent determinant of EI (39). Indeed, a positive
302 association was seen between energy density and EI-energy expenditure ($r = 0.491$; $p <$
303 0.001). When energy density and RMR were included in the same regression module (Table
304 3), both variables were found to independently predict mean daily EI. However, under the
305 conditions of the current study RMR was found to be a stronger predictor of EI than energy
306 density.
307
308 In the present data and that of others (19, 20), no direct relationship was found between FM
309 and EI. These findings are not consistent with the traditional adipocentric view of appetite
310 control. However, they should not be taken to imply that FM does not play a role in appetite
311 regulation. Indeed, a negative association between the FM index and daily EI has been
312 reported (22), which is consistent with an inhibitory role for FM in appetite control.
313 Furthermore, in the path analysis used in the present study indicated that FM indirectly
314 influenced EI via its effect on RMR. Therefore, future research should look to further define
315 how FM, FFM and RMR operate in concert under varying conditions of energy balance.
316 Furthermore, the present findings reflect appetite regulation under conditions close to energy
317 balance in moderately active individuals (1.69 x RMR). They do not therefore provide insight
318 into the mechanisms controlling EI during dynamic periods of energy change. Such
319 distinctions are important as rate and extent of energy deficit and weight loss can alter
320 physical structure and function (e.g. body composition), which in turn may influence EI and

expenditure. Therefore, it is possible that other regulatory signals (such as leptin) may feature more predominantly in appetite control during sustained energy deficit (40).

It has previously been suggested that the energy demand of tissues (such as the liver) might be expressed through tonic hunger signals (35). While not measured in the present study, FFM (21) and RMR (24) have been found to be associated with daily hunger. Interestingly, no such associations were found in obese individuals (41), with the authors suggesting that elevated levels of FM could blunted the orexigenic drive arising from FFM. However, appetite and body weight regulation appears asymmetrical (42), with the inhibitory action of FM weaker at higher levels of adiposity (potentially due to leptin and insulin resistance). Indeed, this attenuation in tonic inhibition with increased FM could contribute to overconsumption in obese individuals, as the drive to eat arising from energy needs, elevated due to a higher RMR, would remain unabated (23). However, the cross-sectional nature of the present study means that inferences cannot be made regarding how systematic changes in body composition or RMR influence EI.

A strength of the present study was the level of precision used to measure EI, energy expenditure and body composition. There was good agreement between the independently assessed components of energy balance, indicating that the procedures used provided a valid measure of EI. As can be seen in figure 1, variability existed in mean daily energy balance. However, while there is a paucity of data on day-to-day variability in energy balance, studies covertly manipulating food or energy expenditure show that such imbalances are not uncommon (39, 43-51). Interestingly, after accounting for RMR, total daily energy expenditure did not explain any further variance in EI. However, total daily energy expenditure was measured during a 14-day residential stay, and therefore is unlikely to reflect

346 'free-living' expenditures (although the mean daily PAL in the present study was 1.69 x
347 RMR). Under conditions where energy expenditure is more variable, the influence of total
348 daily energy expenditure on EI may be stronger (but this effect would not likely be mediated
349 by FFM as individuals exhibit a range of total energy expenditures for a given level of body
350 composition or RMR).
351
352 **CONCLUSIONS**
353 These data indicate that RMR is a strong determinant of EI under conditions where food
354 choice varied freely, and suggests that the energy expenditure associated with RMR may act
355 as a feedback signal that drives habitual food intake at a level proportional to basal energy
356 requirements. In contrast, no such relationship existed between FM and EI, suggesting that
357 the conventional adipocentric model of appetite control should be revised to reflect the
358 infuence of RMR on EI. The influence of RMR, in addition to signals stemming from adipose
359 tissue and gastrointestinal peptides, provides a stronger account of the role of whole-body
360 peripheral signals in human appetite control.
361
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364 SW and the project team (Leona O'reilley and Zoe Fuller) conducted the research. JB
365 proposed the manuscript. CD, GWH, GF, MH and RJS analyzed the data & performed the
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Conflict of Interest

The authors declare no conflicts of interest.

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Figure Legends

Figure 1: Bland and Altman plot illustrating the difference between mean daily energy intake (laboratory weighed food intakes) and energy expenditure (doubly labelled water) against the mean of the two measures (n = 59).

Figure 2: Scatter plots and standardised beta coefficients to illustrate the relationship between energy density on daily energy intake and resting metabolic rate on daily energy intake. Hierarchical multiple regression indicated that together, energy density and resting metabolic rate accounted for 61.7% of the variance in energy intake ($F_{(2, 56)} = 45.140$, $p < 0.001$).

Figure 3: Path diagram for the mediation model with the standardized parameter coefficients for the direct effects of fat mass and fat-free mass on resting metabolic rate and resting metabolic rate on energy intake, the indirect effect of fat mass and fat-free mass on energy intake mediated by resting metabolic rate, and the squared multiple correlations (R^2) for resting metabolic rate and energy intake. The mediation model indicates that the effect of fat mass and fat-free mass on energy intake was fully mediated by resting metabolic rate.

Table 1: Descriptive characteristics of subjects (mean ± SD, range).

	Total Sample (n = 59)		**Men (n = 30)**		**Women (n = 29)**	
	Mean ± SD	**Range (min-max)**	**Mean ± SD**	**Range (min-max)**	**Mean ± SD**	**Range (min-max)**
Age, yrs	42.7 ± 13.6	20.0 - 66.0	42.9 ± 13.1	20.0 - 64.0	42.5 ± 14.3	20.0 - 66.0
Stature, cm	1.7 ± 0.1	1.5 - 1.9	1.8 ± 0.1	1.7 - 1.9	1.7 ± 0.1	1.5 - 1.8
Body Mass, kg	75.9 ± 14.3	51.5 - 120.8	82.7 ± 14.5	56.8 - 120.8	68.9 ± 10.3	51.5 - 96.9
BMI, kg/m^2	26.1 ± 3.8	17.9 - 35.9	26.7 ± 4.0	20.0 - 35.9	25.4 ± 3.5	17.9 - 33.0
Body Fat, %	31.4 ± 7.5	12.7 - 44.8	28.6 ± 7.4	12.7 - 40.1	34.3 ± 6.5	20.6 - 44.8

BMI, body mass index.

Table 2: Mean energy intake, energy expenditure, energy balance and rate of body mass change over days 3-14 of the study (mean ± SD).

	Total Sample (n = 59)	**Men (n = 30)**	**Women (n = 29)**
Mean Daily Energy Intake, MJ/d	11.25 ± 2.76	12.30 ± 2.84	10.17 ± 2.20*
Mean Daily Energy Expenditure, MJ/d	11.05 ± 2.19	12.14 ± 2.04	9.91 ± 1.76*
Resting Metabolic Rate, MJ/d	6.56 ± 1.23	7.20 ± 1.17	5.90 ± 0.91*
Mean Daily PAL	1.69 ± 0.26	1.70 ± 0.28	1.69 ± 0.24
Mean Energy Balance, MJ/d	0.21 ± 2.32	0.15 ± 2.21	0.26 ± 2.48
Mean Body Mass Change, kg/d	-0.015 ± 0.09	-0.019 ± 0.10	-0.011 ± 0.07

PAL, physical activity level.*, significant difference between men and women, as indicated by independent t-tests ($P < 0.05$).

Table 3: Regression coefficients showing the effects of energy density and resting metabolic rate on daily energy intake (Model 1: n = 59), fat mass, fat-free mass and resting metabolic rate on daily energy intake (Model 2: n = 59), and resting metabolic rate and total daily energy expenditure on daily energy intake (Model 3: n = 59).

Model 1				**Model 2**				**Model 3**			
Step 2[1]	**B**			**Step 2**[2]	**B**			**Step 2**[3]	**B**		
	Mean Estimate	**SE**	**ß**		**Mean Estimate**	**SE**	**ß**		**Mean Estimate**	**SE**	**ß**
Constant	-2.295	1.446		**Constant**	1.127	1.579		**Constant**	0.174	1.495	
ED	1.430	0.312	0.386*	**FM**	-0.018	0.035	-0.055	**RMR**	0.001	0.000	0.536*
RMR	0.001	0.000	0.610*	**FFM**	0.013	0.042	0.048	**TDEE**	0.294	0.155	0.235
				RMR	0.002	0.000	0.675*				

To examine the relationships between body composition, energy expenditure and daily energy intake, hierarchical multiple regression was used. B, unstandardized beta coefficient; SE, standard error; β, standardized beta coefficient; ED, energy density; RMR, resting metabolic rate; TDEE, total daily energy expenditure. *$p < 0.001$. [1], $R^2 = 0.617$ for Step 2 ($p < 0.001$). [2], $R^2 = 0.478$ for Step 2 ($p < 0.001$). [3], $R^2 = 0.506$ for Step 2 ($p < 0.001$).