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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.

1 ABSTRACT

2

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

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

9 **Methods:** Fifty nine subjects (30 men: mean body mass index = 26.7 ± 4.0 kg/m²; 29
10 women: mean body mass index = 25.4 ± 3.5 kg/m²) completed a 14 day stay in a residential
11 feeding behaviour suite. During days 1 and 2, subjects consumed a fixed diet designed to
12 maintain energy balance. On days 3-14, food intake was covertly measured in subjects who
13 had *ad libitum* access to a wide variety of foods typical of their normal diets. Resting
14 metabolic rate (respiratory exchange), total daily energy expenditure (doubly labelled water)
15 and body composition (total body water estimated from deuterium dilution) were measured
16 on days 3-14.

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

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

25 INTRODUCTION

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

39

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

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

60 **SUBJECTS AND METHODS**

61 **Subjects**

62 Fifty nine volunteers (30 men and 29 women) were recruited from the Aberdeen area (Table
63 1). Subjects were stratified into three age categories (20-35 years, 36-50 years and 51-65
64 years) and two BMI categories (BMI 20-25 kg/m² and BMI >25 kg/m²). Subjects were non-
65 smokers, free from disease and not taking medication known to effect metabolism or appetite.
66 Menopausal and physical activity status were not included as part of this exclusion criteria.
67 Prior to the start of the study written informed consent was obtained and ethical approval was
68 granted by the Joint Ethical Committee of the Grampian Health Board and the University of
69 Aberdeen. Subjects were informed that the purpose of the study was to examine the
70 relationships between diet and lifestyle.

71 **Table 1 here**

72 **Study Design**

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

90

91 **Procedures**

92 **Resting Metabolic Rate**

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

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

100 **Body Composition**

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

108 **Total Daily Energy Expenditure**

109 Total daily energy expenditure was measured on days 3-14 using doubly labelled water. On
110 the morning of day 3, subjects were woken (07.00 hours), emptied their bladders and
111 weighed. At 09.00 hours, subjects provided a baseline urine sample, which was used
112 alongside two background samples collected during days 1 and 2 to provide information on
113 the pre-dose isotopic enrichment of the subjects' body water pools. Immediately after the
114 09.00 hour sample subjects consumed orally a pre-prepared dose of $^2\text{H}_2^{18}\text{O}$, and 100 ml of tap
115 water to prevent the isotope being lost from the buccal cavity. The dose levels were 0.15 g/kg
116 body mass of a 99% $^2\text{H}_2\text{O}-\text{H}_2\text{O}$ mixture and 1.5 g/kg body mass of a 10.0% $\text{H}_2^{18}\text{O}-\text{H}_2\text{O}$
117 mixture for subjects one to 42 and 44. Dose levels of oxygen 18 were reduced to 0.9 g/kg
118 body mass for the remaining nineteen subjects because of the world shortage in doubly
119 labelled water at the time of the experiment. Following this dose, subjects collected urine
120 samples at 4, 5 and 6 hours post administration to enable the plateau to be individually
121 measured.

122 On days 4-14, subjects provided urine samples at 11.00 hours under supervision and these
123 were frozen (-20°C) until analysis. To calculate energy expenditure, urine samples were used
124 for a multi-point stable-isotope analysis using gas isotope ratio MS. The log transformed data
125 of enrichment by time were extrapolated back to time 0, giving a theoretical enrichment at
126 time 0, which provided information on the individual's size of the body water pool assuming
127 the dilution principle. Isotopic enrichment of the post-dose urine samples was analysed
128 relative to the original background amounts. Pool sizes and flux rates were calculated as
129 described by Coward (29). Energy expenditure was calculated from CO₂ production using the
130 Weir equation (30):

131 • $EE = 4.63CO_2 + 16.49(CO_2/\text{respiratory quotient}),$

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

139 **Energy and Macronutrient Intake**

140 On days 3-14, food intake was covertly and objectively measured in subjects who had *ad*
141 *libitum* access to a wide variety of foods from their normal diet. Food intake was measured
142 overtly by subjects for two, 3-day periods during days 3-14 (with the order randomized).
143 Based on 7-day diet histories and shopping list records collected prior to the start of the study,
144 an inventory of foods and beverages typically consumed by each subject in their normal diet
145 was purchased. If subjects reported an item usually consumed in their habitual diet was
146 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 calculate 24 hour EI, with energy and nutrient content calculated using dietary
160 analysis software (Diet 5, Robert Gordon University, Aberdeen).

161

162 **Statistical Analysis**

163 Data are reported as mean \pm 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

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

174

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

184

185 The assumptions of uni and multivariate normality of errors were assessed by skewness and
186 kurtosis coefficients. There was no severe violation of the normal distribution (33), with
187 skewness values ranging from 0.35 (FM) to 1.07 (EI), and with kurtosis values ranging from
188 0.67 (FFM) to 2.49 (EI). The significance of the direct, indirect and total effects was assessed
189 using Chi-Square tests (33). The Bootstrap resampling method was further used to test the
190 significance of the mediational paths, using 2000 Bootstrap samples and 95% bias-corrected
191 confidence intervals (CI) around the standardized estimates of the effects. Effects were
192 regarded as significantly different from zero ($p < 0.05$) if zero was not included in the interval
193 between the lower and the upper bound of the 95% bias-corrected CI (33). The software
194 AMOS (Analysis of Momentary Structure, software version 18, SPSS Inc. Chicago, IL) was
195 used to estimate the path analysis.

196

197 RESULTS

198 Validation of the Laboratory-Weighed Intakes

199 **Table 2 here**

200

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

- 204
 - $EI = \text{total energy expenditure} \pm \Delta \text{ body stores.}$

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

214

215 Predictors of Daily Energy Intake

216 In order to examine the relationships between body composition, energy expenditure and EI,
217 three separate hierarchical multiple regression models were used (Table 3). In Model 1,
218 energy density was added in the first step ($F_{(1, 57)} = 20.045$, $p < 0.001$), and accounted for
219 26.0% of the variance in daily EI. The addition of RMR (step 2) significantly improved the
220 model ($F_{(2, 56)} = 45.140$, $p < 0.001$; $R^2 = 0.617$), accounting for a further 35.7% of unique
221 variance in EI. During this final step, both energy density ($\beta = 0.390$; $p < 0.001$) and RMR (β
222 $= 0.610$; $p < 0.001$) independently predicted EI (Figure 2).

223

224 In Model 2, step 1 accounted for 29.9% of the variance in daily EI ($F_{(2, 56)} = 11.947$, $p <$
 225 0.001), with FFM ($\beta = 0.514$; $p < 0.001$), but not FM ($\beta = 0.096$; $p = 0.410$), independently
 226 predicting EI. Again, the addition of RMR further improved the model (Step 2; $F_{(3, 55)} =$
 227 16.769 , $p < 0.001$; $R^2 = 0.478$), accounting for an additional 17.9% of unique variance in EI.
 228 During this final step, only RMR independently predicted EI ($\beta = 0.675$; $p < 0.001$).

229

Table 3 here

230 In Model 3, RMR was added in the first step and accounted for 47.4% of the variance in EI
 231 ($F_{(1, 57)} = 51.358$, $p < 0.001$). In step 2 ($F_{(2, 56)} = 28.661$, $p < 0.001$; $R^2 = 0.506$), the addition
 232 of total daily energy expenditure failed to further improve the model ($\Delta R^2 = 0.032$; $p =$
 233 0.063), with RMR the only independent predictor of EI ($\beta = 0.536$; $p < 0.001$). For each
 234 model, age, BMI and sex were also entered in a final Step. However, the addition of these
 235 variables failed to influence the reported outcomes, and therefore, these variables were not
 236 included for analysis in the reported models.

237

Figure 2 here

238 Path Analysis

239 The hypothesised model was tested through a fully saturated model that included 14
 240 parameters. Results indicated that the paths regarding the direct effects of FM on EI ($b_{FM} = -$
 241 0.018 ; $SEb = 0.034$; $Z = -0.529$; $p = 0.597$; $\beta = -0.055$), and FFM on EI ($b_{FFM} = 0.013$; $SEb =$
 242 0.041 ; $Z = 0.331$; $p = 0.740$; $\beta = 0.05$), exceeded the critical value for two-tailed statistical
 243 significance at the 0.05 level (Figure 3). These non-significant paths were removed and the
 244 model was recalculated.

245

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

248 indices: TLI = 1.053; CFI = 1.000; RMSEA = 0.000 ($p = 0.835$). All path coefficients were
249 statistically significant ($p < 0.05$), and the model accounted for 47% of EI variance. Fat mass
250 and FFM were significantly correlated and accounted for 61% of RMR, with a direct effect of
251 0.224 ($b_{FM} = 32.942$; $SEb = 12.526$; $Z = 2.630$; $p = 0.009$) and 0.691 ($b_{FFM} = 88.123$; $SEb =$
252 10.849 ; $Z = 8.123$; $p < 0.001$), respectively. Only RMR presented a significant direct effect (β
253 $= 0.688$) on EI ($b_{RMR} = 0.002$; $SEb = 0.000$; $Z = 7.229$; $p < 0.001$).

254

255 Regarding the mediational tests, results indicated that FM presented an indirect effect of
256 0.154 on EI mediated by increased RMR. Also, FFM predicted increased EI with an indirect
257 effect of 0.476, again through increased RMR. According to the Bootstrap resampling
258 method, the estimates of the indirect effects of FM (CI = 0.045 to 0.278, $p = 0.006$) and FFM
259 (CI = 0.312 to 0.610, $p = 0.001$) on EI, framed by a CI of 0.95%, were significantly different
260 from zero.

261

262 **Figure 3 here**

263 **DISCUSSION**

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

271

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

281

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

296

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 ($1.69 \times \text{RMR}$). 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

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

323

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

336

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

362 **Acknowledgments**

363 The authors' responsibilities were as follows: RJS, GWH and SW conceived the project; RJS,
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
366 statistical analysis. MH, GF, JB and RJS wrote the initial manuscript, while all authors
367 commented on the manuscript. RJS had primary responsibility for final content. The authors
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375 **Conflict of Interest**

376 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.