UNIVERSITY OF LEEDS

This is a repository copy of *Biological and psychological mediators of the relationships between fat mass, fat-free mass and energy intake*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/128578/

Version: Accepted Version

Article:
Hopkins, M orcid.org/0000-0002-7655-0215, Finlayson, G orcid.org/0000-0002-5620-2256, Duarte, C orcid.org/0000-0002-6566-273X et al. (6 more authors) (2019) Biological and psychological mediators of the relationships between fat mass, fat-free mass and energy intake. International Journal of Obesity, 43 (2). pp. 233-242. ISSN 0307-0565

https://doi.org/10.1038/s41366-018-0059-4

© 2018 Macmillan Publishers Limited, part of Springer Nature. This is an author produced version of a paper published in International Journal of Obesity. Uploaded in accordance with the publisher's self-archiving policy.

Reuse
Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

White Rose university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

AUTHOR VERSION

Author Version: Published ahead of online first

Biological and psychological mediators of the relationships between fat mass, fat-free mass and energy intake

Mark Hopkins, Graham Finlayson, Cristiana Duarte, Catherine Gibbons, Alexandra M Johnstone, Stephen Whybrow, Graham W Horgan, John E Blundell, R James Stubbs

Cite this article as: Mark Hopkins, Graham Finlayson, Cristiana Duarte, Catherine Gibbons, Alexandra M Johnstone, Stephen Whybrow, Graham W Horgan, John E Blundell and R James Stubbs, Biological and psychological mediators of the relationships between fat mass, fat-free mass and energy intake, *International Journal of Obesity* _####################_ doi:10.1038/s41366-018-0059-4

This is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication. Springer Nature are providing this early version of the manuscript as a service to our customers. The manuscript will undergo copyediting, typesetting and a proof review before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers apply.

Received 07 November 2017; accepted 09 February 2018;
Author version _####################_

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Biological and psychological mediators of the relationships between fat mass, fat-free mass and energy intake

Mark Hopkins[1], Graham Finlayson[2], Cristiana Duarte[2], Catherine Gibbons[2], Alexandra M Johnstone[3], Stephen Whybrow[3], Graham W Horgan[4], John E Blundell[2] and R James Stubbs[2].

[1]School of Food Science and Nutrition, Faculty of Mathematics and Physical Sciences, University of Leeds, Leeds, United Kingdom. [2]Institute of Psychological Sciences, Faculty of Medicine and Health, University of Leeds, Leeds, United Kingdom. [3]Rowett Institute, University of Aberdeen, Aberdeen, United Kingdom. [4]Biomathematics and Statistics Scotland, Aberdeen, United Kingdom.

Corresponding author:

Dr Mark Hopkins

School of Food Science and Nutrition,

Faculty of Mathematics and Physical Sciences,

University of Leeds,

Leeds,

United Kingdom.

Tel: +44 (0) 11334 36990

Email: M.Hopkins@Leeds.ac.uk

Running heading: Fat mass, fat-free mass & energy intake

Disclosure of funding:

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

The present study was funded by the Food Standards Agency, UK, and The Scottish Government's Rural and Environment Science and Analytical Services Division. None of the funding bodies had a role in the design, analysis or writing of this article.

Disclaimers:

The authors declare no conflicts of interest.

Abbreviations:

FFM, fat-free mass; FM, fat mass; RMR, resting metabolic rate; EI, energy intake; EE, energy expenditure; HR; heart rate; DEBQ_R, restraint sub-score from the Dutch Eating Behaviour Questionnaire. DEBQ_EM, emotional eating sub-score from the Dutch Eating Behaviour Questionnaire; DEBQ_Ext, external eating sub-score from the Dutch Eating Behaviour Questionnaire.

Names for PubMed Indexing:

Hopkins, Finlayson, Duarte, Gibbons, Johnstone, Whybrow, Horgan, Blundell, Stubbs.

This trial was registered at clinicaltrials.gov as NCT03319615.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

ABSTRACT

Background: While recent studies indicate that in humans fat-free mass (FFM) is closely associated with energy intake (EI) when in energy balance, associations between fat mass (FM) and EI are inconsistent.

Objectives: The present study used a cross-sectional design to examine the indirect and direct effects of FFM, FM and resting metabolic rate (RMR) on EI in individuals at or close to energy balance.

Methods: Data for 242 individuals (114 males; 128 females; BMI = 25.7 ± 4.9 kg/m^2) were collated from the non-intervention baseline conditions of five studies employing common measures of body composition (air displacement plethysmography), RMR (indirect calorimetry) and psychometric measures of eating behaviours (Dutch Eating Behaviour Questionnaire). Daily EI (weighed-dietary records) and energy expenditure (flex heartrate) were measured for 6-7 days. Sub-analyses were conducted in 71 individuals who had additional measures of body composition (dual-energy X-ray absorptiometry) and fasting glucose, insulin and leptin.

Results: After adjusting for age, sex and study, linear regression and mediation analyses indicated that the effect of FFM on EI was mediated by RMR ($P < 0.05$). FM also independently predicted EI, with path analysis indicating a positive indirect association (mediated by RMR; $P < 0.05$), and a stronger direct negative association ($P < 0.05$). Leptin, insulin and insulin resistance failed to predict EI, but cognitive restraint was a determinant of EI and partially mediated the association between FM and EI ($P < 0.05$).

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Conclusions: While the association between FFM and EI was mediated by RMR, FM influenced EI via two separate and opposing pathways; an indirect 'excitatory' effect (again, mediated by RMR), and a stronger direct 'inhibitory' effect. Psychological factors such as cognitive restraint remain robust predictors of EI when considered alongside physiological determinants of EI, and indeed, have the potential to play a mediating role in the overall expression of EI.

KEY WORDS

Energy intake, appetite regulation, body composition, fat mass, fat-free mass, resting metabolic rate, energy expenditure, energy balance.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

INTRODUCTION

Despite substantial interest into the putative causes of weight gain and obesity, fundamental questions remain over the nature and extent of the biological regulation of human energy intake (EI), and the relationship between physiology and behaviour in determining energy balance. While understanding of the putative peripheral signals that affect EI has improved, this has not yet yielded a means to prevent weight gain or promote weight loss maintenance. As such, there has been renewed interest in integrative models of weight gain and loss using energy balance methodology, as this provides an opportunity to integrate physiological and behavioral determinants of appetite with dynamic changes in body structure and function.

Recent studies have demonstrated that fat-free mass (FFM) is more strongly associated with EI than fat mass (FM) in those at or close to energy balance,[1-6] with FFM 'indirectly' influencing EI through the energetic demands of metabolically active tissue.[3, 4] However, while the associations between body composition, EE and EI have been demonstrated under controlled laboratory conditions, it remains unclear whether FFM and RMR are strong determinants of EI under free-living conditions where EI is influenced by multiple social and environmental factors.[7] Furthermore, in contrast to the consistent associations between FFM and EI, negative[1, 4, 8] or no associations[2, 5, 6, 9] have been reported between FM and EI at or close to energy balance. A negative association between FM and EI is consistent with the proposed inhibitory role of FM (and leptin) in appetite control,[10] but such feedback is inconsistent with the apparent ease with which humans can gain weight.

Psychological factors may also mediate the effects of FM on EI, but the conjoint influence of biological and psychological factors on EI is rarely examined. McNeil et al.[11] recently reported that the combination of RMR and prospective food consumption explained a greater proportion of variance in daily EI than RMR alone (n = 55). However, whether psychological

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

factors directly mediate the associations between FM, FFM and RMR and EI has not been examined. Therefore, the aim of this study was to examine the specific indirect and direct effects of FM, FFM and RMR on EI in individuals at or close to energy balance, and whether any associations between FM and EI were mediated by leptin, insulin or psychometric eating behaviours.

SUBJECTS & METHODS

Subjects

In total, 242 subjects (114 males; 128 females; BMI = 25.6 ± 5.0 kg/m^2; Table 1) were included in the present analysis, with data aggregated from the control conditions of five separate studies with common experimental procedures. A flow chart detailing the participant contribution from each study can be found in the online supplementary material (Supplementary Figure 1). All data were collected at the Rowett Institute, University of Aberdeen, UK between 1998 and 2007, and aspects of these data have been published previously.[12-17] The individual studies were originally designed to examine the effects of diet on body composition and health, and subjects were informed that their purpose was to examine the relationships between diet and lifestyle. For each 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 weight stable (<2 kg change in the previous three months), free from disease and not taking medication known to effect metabolism or appetite. The present study was registered at clinicaltrials.gov as NCT03319615.

Table 1 here

Study Design

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

The present study examined the cross-sectional associations between body composition (air displacement plethysmography), RMR (indirect calorimetry) and total daily EI (weighed dietary records) and EE (flex heart rate; HR). Data were aggregated from the non-intervention, baseline control conditions of five separate studies that employed common experimental procedures, with daily EI and EE measured over six (n = 54) or seven (n = 188) days. Detailed descriptions of the procedures used, repeatability of measurements, and the assumptions and limitations associated with these data can be found elsewhere.[12, 13, 16, 18-22]

Anthropometry and Body Composition

Baseline body weight was measured to the nearest 0.01 kg after voiding in all subjects (DIGI DS-410 CMS Weighing Equipment, London, UK), while the change in body weight over the measurement period was measured in 229 subjects. In each case, subjects were weighed in dressing gowns of a known weight, with body weight then corrected back to nude weight. Stature was measured to the nearest 0.5 cm using a portable stadiometer (Holtain Ltd., Crymych, Dyfed, Wales).

Body composition was estimated using air-displacement plethysmography (BOD POD Body Composition System, Life Measurement, Inc., Concord, USA) in 233 subjects. Measurements were taken according to manufacturers' instructions while wearing minimal clothing, with thoracic gas volumes estimated using the manufacturer's software. This technique has been validated against underwater weighing in normal[23] and overweight and obese adults.[24] In a nine subjects, body composition was estimated from skinfold thickness (Holtain Ltd., Dyfed, Wales, UK) and the equations of Durnin & Womersley[25] as measures of air-displacement plethysmography were unavailable. The inclusion of these subjects alongside those with estimates using air-displacement plethysmograph did not alter the outcomes of any analyses.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Resting Metabolic Rate

RMR was measured by indirect calorimetry over 30-40 minutes using a ventilated hood system (Deltatrac II, MBM-200, Datex Instrumentarium Corporation, Finland). Following a 12 hour fast, subjects laid on a bed in a thermo-neutral room and were instructed to lie still but remain awake. Resting EE was calculated from minute-by-minute data using the mean of 15 minutes of stable measurements, with the first and last five minutes excluded. The equations of Elia and Livesey[21] were used to derive RMR. Details of calibration burns and repeatability testing have been described previously.[17]

Daily Energy Intake

Energy intake was measured using a weighed dietary record method in which subjects recorded all foods and drinks consumed for either six or seven days. Full written and verbal information on how to complete the record was given at the beginning of each study. Subjects were provided with calibrated digital electronic scales to a resolution of 1 g (820 Soehnle, Soehnle-Waagen GmbH & Co. KG, Murrhardt, Germany), and a food diary for recording of food/drink, time of consumption, food weight, cooking method and leftovers. Subjects were encouraged to record all recipe formulations and to keep all packaging for ready-to-eat food products. When scale use was difficult (i.e. when eating out), subjects were instructed to record as much information as possible about the quantity of the food they ate by using household measures (e.g. tablespoon, cup, slice). Data were analysed using Diet 5 (Robert Gordon University, Aberdeen), which was updated for unusual food products based on the food packaging provided by subjects. Standard portions sizes were used with missing weights or portion sizes, and to reduce investigator bias and inputting errors, all diets were cross-checked by at least one other trained member of staff.

Psychometric Eating Behaviours

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

The Dutch Eating Behaviour Questionnaire[26] was administered in 193 subjects to assess trait levels of cognitive restraint, emotional eating behaviour and external eating behaviour. The Dutch Eating Behaviour Questionnaire is a 33-item questionnaire that uses a 5-point Likert scale ranging from 1 (seldom) to 5 (very often) to assess three eating behaviour domains: the restrained subscale (10 items- DEBQ_R), the emotional eating subscale (13 items- DEBQ_EM), and the external eating subscale (10 items- DEBQ_Ext). The questionnaire has previously been found to have good psychometric properties.[26]

Total Daily Energy Expenditure

To examine the validity of the EI measures in the present study, energy balance (i.e EI - EE) was compared to the change in body weight over the measurement period. Mean daily EE was calculated using the modified flex HR method of Ceesay et al.,[20] and the calorimetric equations of Elia and Livesey.[27] Total daily EE was calculated from a minimum of 12 hours of HR data per day (Polar Sport Tester, Polar Electro Oy, Finland). HR was averaged over 1-minute intervals throughout the waking day, with subjects recording the time at which they started and stopped wearing the HR monitors each day. A regression line of HR vs. EE was established for each subject by simultaneously measuring HR, breath-by-breath $\dot{V}O_2$ and $\dot{V}CO_2$ (averaged over 10-s intervals) at incremental workloads in the morning following an overnight fast. As previously described,[28] the test comprised of a series of sedentary activities and an incremental cycle test in the following sequential steps with no break between them: 5 min sitting, 5 min standing up, 5 min cycling at the lowest possible resistance (55 W), and a further 3 × 5-min blocks increasing resistance and maintaining 60 rpm. The average of the two calibration curves was used for calculation of EE, with daily EE was estimated from:

- Total daily EE = sedentary EE + sleep EE + activity EE [20, 29]

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Sleep EE was calculated as 95% of measured RMR[30] and was applied to the time when the HR monitors were not worn (i.e. during sleep). Sedentary EE was assumed to be equal to the mean EE from RMR, sitting, and standing measurements during the calibration.[29] However, as these measures were performed following an overnight fast, the thermic effect of food would not have been accounted for in these calculations, and this would have likely resulted in an under-estimation of total daily EE in the present study. For HR exceeding flex HR, HR was calculated using the treatment-specific HR: O_2 calibration regression equation for each individual. Zero values and heart rates that were considered to be outside of the physiological range (>220 beats/min) were removed and replaced by the average of the previous and subsequent values.[31]

Sub-Analysis

A sub-analysis was conducted in 71participants who had additional measures of body composition (dual-energy X-ray absorptiometry) and fasting glucose, insulin and leptin. These were also included in the main analysis, and RMR, EE and EI were measured using the above procedures. Body composition was assessed using a Norland XR-26, Mark II high-speed pencil beam scanner equipped with dynamic filtration (version 2.5.2 of the Norland software; Norland Corporation, Fort Atkinson, WI) following an overnight fast. Fasted whole blood was also taken from an antecubital vein and collected into a 10-mL lithium heparin tube and spun in a chilled centrifuge (1000 g at 4 °C for 10 min) to obtain plasma and stored at 80 °C for batch analysis. Plasma leptin was measured using radioimmunoassay (BioVendor GmbH, Heidelberg, Germany), while plasma insulin was measured using enzyme-linked immunosorbent assay (LINCO Research, St Charles, Missouri, USA). Insulin resistance

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

(HOMA IR) was calculated using the homeostatic model of assessment[32] based on the fasting measures of glucose and insulin.

Statistical Analysis

Statistical analyses were performed using IBM SPSS for windows (Chicago, Illinois, Version 24), and data are reported as mean ± SD. A paired t-test was used to examine for differences between mean daily EI and EE. A Bland and Altman plot was used to compare the deviations between the methods used for the assessment of energy balance. Based on previous research findings,[3, 4, 33] two regression models were constructed using general linear modelling with EI as the dependent variable. In model one, FM, FM and RMR were entered as independent variables (n = 242). In model two, DEBQ_R, DEBQ_EM and DEBQ_Ext were also entered as independent variables alongside FM, FFM and RMR (n = 193). A 'study' term was included in both models to account for heterogeneity between separate studies, and given their known effects on RMR and EI, sex and age were also included in both models. In a sub-sample of data (n = 71), linear regression was performed with EI as the dependent variable and sex, age, FM, FFM, RMR, and one of leptin, insulin or HOMA_IR included as independent variables. Multicollinearity was assessed using the variance inflation factor (VIF), which indicated that there was no instability in any of the models (with VIF scores below 7.0 for all predictors included in the regression models).[34]

Path analysis was used to further examine the associations between age, sex, FM, FFM, RMR, DEBQ_R, and EI. A model initially tested whether the associations between sex and the standardised residual scores (after adjusting for study using residuals from a linear regression model which had a term for study only) of age, body composition (independent, exogenous variables) and EI (dependent, endogenous variable) were mediated by RMR

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

(endogenous mediator variable). A second model was also tested examining whether the addition of the standardised residual score (after adjusting for study) for dietary restraint altered the associations between the standardised residual scores of age, body composition (independent, exogenous variables), RMR (endogenous mediator variable) and EI (dependent, endogenous 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.[34, 35] Indirect effects were tested through the bootstrapping method, with 2000 Bootstrap samples and 95% bias-corrected confidence intervals (CI). Effects were significant when zero was not included in the CI lower and upper limits.[34, 35]

RESULTS

Table 2 here.

Mean daily EI, EE, energy balance and the change in body weight can be seen in Table 2. There was a significant difference between EI and EE, producing a mean energy deficit of -1250 kJ/d ($P < 0.01$). The relationship between EI and EE was also plotted as a Bland and Altman diagram to illustrate the spread of the differences (EI-EE) against the mean of the two methods (Figure 1). Overall, there was a good spread in the data with no apparent trend. However, the intercept of the average weight change and energy balance was found to differ significantly from zero (coefficient = -0.401; SE = 0.064; $P < 0.001$).

Figure 1 here

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Influence of Body Composition, Energy Expenditure and Psychometric Eating Behaviours on Food Intake

After accounting for sex (ß = 0.12; P = 0.247), age (ß = -0.08; P = 0.184) and study (P = 0.024 to P = 0.490) in model one ($F_{(9, 232)}$ = 18.85, P < 0.001; R^2 = 0.42- Table 3), RMR (ß = 0.39; P = 0.001) and FM (ß = -0.29; P <0.001) independently predicted EI. In model two ($F_{(11, 193)}$ = 15.16, P < 0.001; R^2 = 0.48), RMR (ß = 0.30; P = 0.008) and DEBQ_R (ß = -0.26; P < 0.001) independently predicted EI after accounting for sex (ß = 0.09; P = 0.395), age (ß = 0.10; P = 0.139) and study (P = 0.064 to P = 0.465).

Table 3 here

Influence of Leptin and Insulin on Energy Intake (n = 71)

While associations between FM, FFM, RMR and EI were similar to that reported above, leptin ($F_{(6, 64)}$ = 8.39, P < 0.001; R^2 = 0.44; ß = 0.02; P = 0.833), insulin ($F_{(6, 64)}$ = 8.50, P < 0.001; R^2 = 0.44; ß = 0.07; P = 0.515) or HOMA_IR ($F_{(6, 64)}$ = 7.24, P < 0.001; R^2 = 0.40; ß = 0.15; P = 0.582) were not independent predictors of EI.

Path Analysis- Body Composition and Resting Metabolic Rate

To further explore the associations reported in regression models one and two, the mediator effect of RMR was initially examined using path analysis (Figure 2a). Sex and standardised residual scores of FM, FFM, RMR, age and EI were used in the model after adjusting for study. The model was first examined through a fully saturated model with 29 parameters. Results showed that the path relating the direct effect of FFM on EI was non-significant (b_{FFM} = .18; SEb = .11; Z = 1.60; P = 0.109; β = 0.18). The effects of sex (b_{sex} = .15; SEb = .16; Z = -0.92; P = 0.359; β = -0.07) and age (b_{age} = -.07; SEb = .06; Z = -1.25; P = 0.210; β = 0.18) on

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

EI were also non-significant. The recalculated model with the non-significant paths removed presented a very good model fit ($\chi^2_{(3)}$ = 6.13, P = 0.106; TLI = 0.98; CFI = 1.00; RMSEA = 0.07, P = 0.285). The model accounted for 78% of RMR and 36% of EI variance.

FM (β_{FM} = 0.40; b_{FM} = 0.40; SEb = 0.03; Z = 11.87; P < 0.001) and FFM (β_{FFM} = 0.68; b = 0.68; SEb = 0.05; Z = 14.36; P < 0.001) presented a significant direct effect on RMR. RMR in turn, presented a significant direct effect on EI (β_{RMR} = 0.; b = 0.63; SEb = 0.06; Z = 11.20; P < 0.001). FM had a significant total effect on EI (β_{FM} = -0.16), with a direct effect of -0.41 and an indirect effect of 0.25 mediated by RMR. FFM predicted increased EI with an indirect effect of 0.43, fully mediated by RMR. The estimates of the indirect effect of FM (CI = 0.19, 0.33, P = 0.33) and FFM (CI = 0.35, 0.51, P = 0.001), on EI, framed by a CI of 0.95% were significantly different from zero. Age and sex presented a significant direct effect on RMR (β_{age} = -0.18; b_{age} = -0.18; SEb = 0.03; Z = -5.53; P < 0.001; β_{sex} = -0.12; b_{sex} = -0.23; SEb = 0.09; Z = -2.47; P = 0.014) and an indirect effect on EI of -0.11 (CI_{age} = -0,16, 0.08, P = 0.001) and -0.07 (CI_{sex} = -0.13, -0.01, P = 0.010), respectively.

Path Analysis- Body Composition, Resting Metabolic Rate and Dietary Restraint

An additional model that considered the mediator effect of DEBQ_R was examined (Figure 2b) as DEBQ_R was found to be a significant predictor of EI in regression model two. Sex and standardised residual scores of FM, FFM, RMR, DEBQ_R, age and EI were used in the model after adjusting for study. The model presented a very good model fit ($\chi^2_{(6)}$ = 13.38, P = 0.37; TLI = 0.96; CFI = 0.99; RMSEA = 0.08, P = 0.167). The model accounted for 75% of RMR, 17% of DEBQ_R and 39% of EI variance. DEBQ_R presented a significant direct negative association with EI (β_{DEBQ} = -0.25; b = -0.27; SEb = 0.06; Z = -4.41; P < 0.001). Results indicated that FM had a significant indirect effect of 0.14 on EI (CI = 0.05, 0.23, P =

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

0.006), mediated by RMR with an effect of 0.22 and by DEBQ_R with an effect of -0.09. The associations between the other variables maintained the same direction and strength. FFM presented a significant indirect effect on EI of 0.38, fully mediated by RMR (CI = 0.30, 0.47, P = 0.01).

DISCUSSION

This study examined the specific indirect and direct effects of FM, FFM and RMR on EI in a large and heterogeneous sample. The present data indicate that FFM is a strong determinant of self-recorded weighed EI. However, mediation analysis revealed the effect of FFM on EI was mediated by RMR, such that FFM did not statistically influence EI independent of its effect on EE. In contrast, FM influenced EI via two associations that appeared to follow separate and opposing pathways; an indirect excitatory effect mediated via RMR and a stronger direct inhibitory effect (although the strength of this direct association was still weaker than that seen between RMR and EI). While leptin, insulin or HOMA IR did not predict EI, cognitive restraint was found to predict EI and partially mediated the direct association between FM and EI.

Fat-Free Mass, Resting Metabolic Rate and Energy Intake

Consistent with previous findings under laboratory conditions,[1-6] FFM was found to predict self-recorded weighed EI under conditions more representative of the free-living environment. However, mediation analysis revealed the effect of FFM on EI was mediated by RMR. The effect of FFM on EI has previously been attributed to its contribution to EE, with associations between FFM and EI previously reported to be mediated by RMR[3] and 24-hour EE.[4] Taken together, these data suggest that the energetic demand created by FFM acts as a tonic driver of EI under conditions of approximate energy balance. However, in light of the

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

emergence of skeletal tissue as an important endocrine organ,[36] a direct molecular pathway linking FFM to EI that operates independent of EE should not be dismissed (particularly under conditions when functional stores of FFM are challenged).[9, 37] As such, there is a need to examine the peripheral and central putative mechanisms that link FFM and EE to EI.

Fat Mass and Food Intake

In the present study, FM was associated with EI via two separate and opposing pathways; a weak indirect positive association (mediated via RMR) and a stronger direct negative association. While these direct and indirect associations represent statistical rather than biological pathways, they are consistent with the proposed effects of FM on RMR and EI. In line with the smaller contribution of FM to RMR,[17, 38] the indirect effect of FM on EI (mediated by RMR) was weaker than that for FFM. Similarly, the direct negative association between FM and EI (independent of RMR) is consistent with the proposed inhibitory role for FM in appetite control i.e. that increases in FM, and in turn, leptin, promote reductions in hunger and EI via alterations in the expression of anorexigenic and orexigenic neuropeptides in the arcuate nucleus of the hypothalamus.[10] However, despite extensive literature on leptin and other putative feedback signals arising from adipose tissue,[39, 40] there appears limited evidence in humans that FM exerts strong negative feedback on EI under conditions of approximate energy balance (or indeed, energy surfeit). In line with this, the strength of the negative (inhibitory) association between FM and EI in the present study was weaker than the positive (excitatory) association between RMR and EI. This mis-match between inhibitory and excitatory associations may have important implications for overconsumption, with the balance between these opposing drives influencing the overall expression of appetite and EI. A number of previous studies examining the role of FM on EI, including those of our own, have reported no association between FM and EI under conditions of approximate energy

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

322 balance.[2, 5, 6, 9] However, the present study employed a larger sample than previous studies (n
323 = 242), potentially increasing our ability to detect a weaker, but physiological relevant,
324 association.

325 It has been suggested that FM influences EI via the tonic action of leptin and insulin.[10] While
326 leptin appears to be a key central putative appetite signal,[10] evidence that FM or peripheral
327 leptin concentrations exert strong negative feedback on day-to-day feeding under conditions
328 of energy balance is limited. In line with this, leptin, insulin or HOMA IR predicted EI in the
329 present study, suggesting that the 'direct' association seen between FM and EI was not
330 biologically mediated (although this analysis was performed in a small sample of individuals
331 free from insulin resistance, and other potential hormonal mediators clearly exist). In contrast,
332 cognitive restraint predicted EI and partially mediate the direct association between FM and
333 EI. Cognitive restraint can be viewed as an enduring trait that manifests itself as a conscious
334 or subconscious pressure to reduce EI,[26] and this type of function would account for the
335 inverse association between DEBQ_R and EI seen in the present study. There is also evidence
336 that restraint is positively associated with BMI, with individuals with high BMIs tending to
337 show higher levels of restraint (as restraint is a self-reported measure of attempted EI
338 restriction rather than an actual measure of success).[41] The 'encoding' of restraint in biology
339 is not known and is likely to be complicated. However, it is plausible that FM is one of a
340 number of predictors of restraint, and that restraint is one of the pathways that mediates the
341 negative effect of FM on EI. The present findings indicate that psychological factors such as
342 cognitive restraint remain robust predictors of EI when considered alongside physiological
343 determinants. However, few studies have sought to integrate determinants from differing
344 scientific domains, and this has limited our understanding of how physiological,
345 psychological and behavioural factors interact in a co-ordinated fashion within an energy
346 balance framework.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Despite common methodological procedures, aggregation of data from separate studies will have introduced heterogeneity. Therefore, a study term was included in all statistical models (and accounted for ≈5% of variance in EI). These data are cross-sectional and correlational in nature, and do not provide evidence into the mechanisms that drive EI during significant weight loss or gain. However, they do provide a framework for considering how such mechanisms may operate. Given the limitations associated with self-report EI[7] and flex HR,[29] we compared EI and EE to change in body weight as an independent index of energy balance. This indicated that on average individuals were in an energy deficit and a detectable bias exited in measured energy balance compared to the change in body weight. This bias may have resulted from an underestimation of EI due to dietary mis-reporting,[7] and/or an underestimation in total daily EE as the thermic effect of food was not specifically accounted for.[29] In comparison to our previous paper where FM, FFM & RMR accounted for 47% of the variance in EI under laboratory conditions,[3] in the present study these variables only accounting for ≈37% of the variance in self-recorded EI. This likely reflects differences in the methods used to measure EI, but there is no evidence that the bias in EI-EE compared to the change in body weight influenced the overall patterns in any of the models calculated in the present paper. Indeed, despite the additional 'noise' introduced by current approach, strong associations were still seen between FFM, RMR and EI. Furthermore, we show that models integrating physiological and psychometric factors explain a greater proportion of the variance in EI.

Accepted manuscript

CONCLUSIONS

These data indicate that FFM is a strong determinant of EI under conditions of approximate energy balance, with its effect mediated by RMR. FM influenced EI via two associations that were weaker and appeared to follow separate and opposing pathways, highlight the

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

importance of examining the balance between inhibitory and excitatory signals from specific tissues when trying to understand the determinants of EI. Psychological factors such as cognitive restraint remain robust predictors of EI when considered alongside these physiological determinants of EI, and indeed, have potential to play a mediating role.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

ACKNOWLEDGMENTS

The authors' responsibilities were as follows: RJS, GWH, AMJ and SW conceived the project; RJS, SW, AMJ and the project team (Leona O'Reilley and Zoe Fuller) conducted the research. CD, GWH, MH and RJS analysed the data & performed the statistical analysis. MH, GF, CG, JB and RJS wrote the initial manuscript, while all authors commented on the manuscript. RJS had primary responsibility for final content. The authors report no personal or financial conflicts of interest.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

REFERENCES

1. Weise C, Hohenadel M, Krakoff J, Votruba S. Body composition and energy expenditure predict ad-libitum food and macronutrient intake in humans. Int J Obesity. 2013; **38**(2)**:** 243–251.

2. Lissner L, Habicht J-P, Strupp BJ, Levitsky D, Haas JD, Roe D. Body composition and energy intake: do overweight women overeat and underreport? Am J Clin Nutr. 1989; **49**(2)**:** 320-325.

3. Hopkins M, Finlayson G, Duarte C, Whybrow S, Horgan GW, Blundell J et al. Modelling the Associations between Fat-free Mass, Resting Metabolic Rate and Energy Intake in the Context of Total Energy Balance. Int J Obesity. 2015; **40**(2)**:** 312-8.

4. Piaggi P, Thearle MS, Krakoff J, Votruba SB. Higher daily energy expenditure and respiratory quotient, rather than fat free mass, independently determine greater ad libitum overeating. J. Clin. Endocrinol. Metab. 2015**:** jc. 2015-2164.

5. Caudwell P, Finlayson G, Gibbons C, Hopkins M, King N, Naslund E et al. Resting metabolic rate is associated with hunger, self-determined meal size, and daily energy intake and may represent a marker for appetite. Am J Clin Nutr. 2013; **97**(1)**:** 7-14.

6. Blundell J, Caudwell P, Gibbons C, Hopkins M, Naslund E, King N et al. Body composition and appetite: fat-free mass (but not fat-mass or BMI) is positively associated with self-determined meal size and daily energy intake in humans. Brit J Nutr. 2012; **107:** 445-459.

7. de Castro JM. Eating behavior: lessons from the real world of humans. Nutr. 2000; **16**(10)**:** 800-813.

8. Cugini P, Salandri A, Cilli M, Ceccotti P, Di Marzo A, Rodio A et al. Daily hunger sensation and body composition: I. Their relationships in clinically healthy subjects. Eat Weight Disord. 1998; **3**(4)**:** 168-72.

9. Cameron JD, Sigal RJ, Kenny GP, Alberga AS, Prud'Homme D, Phillips P et al. Body Composition and Energy Intake—Skeletal Muscle Mass is the Strongest Predictor of Food Intake in Obese Adolescents: The HEARTY Trial. Appl Physiol Nutr Metab. 2016. **41**(6):611-7.

10. Schwartz MW, Seeley RJ, Zeltser LM, Drewnowski A, Ravussin E, Redman LM et al. Obesity Pathogenesis: An Endocrine Society Scientific Statement. Endocr Rev. 2017. **38**(4):267–296.

11. McNeil J, Lamothe G, Cameron JD, Riou M-È, Cadieux S, Lafrenière J et al. Investigating predictors of eating: is resting metabolic rate really the strongest proxy of energy intake? Am J Clin Nutr. 2017**:** ajcn153718.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

12. Stubbs RJ, O'Reilly LM, Whybrow S, Fuller Z, Johnstone AM, Livingstone MBE et al. Measuring the difference between actual and reported food intakes in the context of energy balance under laboratory conditions. Brit J Nutr. 2014; **111**(11)**:** 2032-2043.

13. Whybrow S, Stubbs R, Johnstone A, O'reilly L, Fuller Z, Livingstone M et al. Plausible self-reported dietary intakes in a residential facility are not necessarily reliable. Eur J Clin Nutr. 2016; **70**(1)**:** 130-135.

14. Whybrow S, Harrison CL, Mayer C, Stubbs RJ. Effects of added fruits and vegetables on dietary intakes and body weight in Scottish adults. Brit J Nutr. 2006; **95**(03)**:** 496-503.

15. Whybrow S, Mayer C, Kirk TR, Mazlan N, Stubbs RJ. Effects of two weeks' mandatory snack consumption on energy intake and energy balance. Obesity. 2007; **15**(3)**:** 673-685.

16. Fuller Z, Horgan G, O'reilly L, Ritz P, Milne E, Stubbs R. Comparing different measures of energy expenditure in human subjects resident in a metabolic facility. Eur J Clin Nutr. 2008; **62**(4)**:** 560-569.

17. Johnstone AM, Murison SD, Duncan JS, Rance KA, Speakman JR. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. Am J Clin Nutr. 2005; **82**(5)**:** 941-948.

18. Stubbs R, Sepp A, Hughes D, Johnstone A, King N, Horgan G et al. The effect of graded levels of exercise on energy intake and balance in free-living women. Int J Obesity 2002; **26**(6)**:** 866-869.

19. Stubbs R, Sepp A, Hughes D, Johnstone A, Horgan G, King N et al. The effect of graded levels of exercise on energy intake and balance in free-living men, consuming their normal diet. Eur J Clin Nutr. 2002; **56**(2)**:** 129-140.

20. Ceesay SM, Prentice AM, Day KC, Murgatroyd PR, Goldberg GR, Scott W et al. The use of heart rate monitoring in the estimation of energy expenditure: a validation study using indirect whole-body calorimetry. Brit J Nutr. 1989; **61**(02)**:** 175-186.

21. Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. World Rev Nutr Diet. 1991; **70:** 68-131.

22. Murgatroyd P, Shetty P, Prentice A. Techniques for the measurement of human energy expenditure: a practical guide. Int J Obes Relat Metab Disord. 1993; **17**(10)**:** 549-568.

23. Fields DA, Goran MI, McCrory MA. Body-composition assessment via air-displacement plethysmography in adults and children: a review. Am J Clin Nutr. 2002; **75**(3)**:** 453-467.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

24. Ginde SR, Geliebter A, Rubiano F, Silva AM, Wang J, Heshka S et al. Air Displacement Plethysmography: Validation in Overweight and Obese Subjects. Obesity. 2005; **13**(7)**:** 1232-1237.

25. Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Brit J Nutr. 1974; **32**(01)**:** 77-97.

26. Van Strien T, Frijters JE, Bergers G, Defares PB. The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. Int J Eat Disord. 1986; **5**(2)**:** 295-315.

27. Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. In: Metabolic Control of Eating, Energy Expenditure and the Bioenergetics of Obesity. Karger Publishers, 1992, pp 68-131.

28. Stubbs R, Hughes D, Johnstone A, Whybrow S, Horgan G, King N et al. Rate and extent of compensatory changes in energy intake and expenditure in response to altered exercise and diet composition in humans. Am J Physiol Regul Integr Comp Physiol. 2004; **286**(2)**:** 350.

29. Spurr G, Prentice A, Murgatroyd P, Goldberg G, Reina J, Christman N. Energy expenditure from minute-by-minute heart-rate recording: comparison with indirect calorimetry. Am J Clin Nutr. 1988; **48**(3)**:** 552-559.

30. Goldberg G, Prentice A, Davies H, Murgatroyd P. Overnight and basal metabolic rates in men and women. Eur J Clin Nutr. 1988; **42**(2)**:** 137-144.

31. Wareham NJ, Hennings SJ, Prentice AM, Day NE. Feasibility of heart-rate monitoring to estimate total level and pattern of energy expenditure in a population-based epidemiological study: the Ely Young Cohort Feasibility Study 1994–5. Brit J Nutr. 1997; **78**(06)**:** 889-900.

32. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; **28**(7)**:** 412-419.

33. Blundell JE, Caudwell P, Gibbons C, Hopkins M, Naslund E, King N et al. Role of resting metabolic rate and energy expenditure in hunger and appetite control: a new formulation. Dis. Models Mech. 2012; **5**(5)**:** 608-613.

34. Hair JF, Tatham RL, Anderson RE, Black W. Multivariate data analysis, vol. 6. Pearson Prentice Hall: New Jersey, 2006.

35. Kline RB. Principles and practice of structural equation modeling, Guilford press: New York, 2005.

36. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat. Rev. Endocrinol. 2012; **8**(8)**:** 457-465.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

37. Weise CM, Thiyyagura P, Reiman EM, Chen K, Krakoff J. A potential role for the midbrain in integrating fat-free mass determined energy needs: An H215O PET study. Hum. Brain Mapp. 2015; **36**(6)**:** 2406-2415.

38. Johnstone A, Rance K, Murison S, Duncan J, Speakman J. Additional anthropometric measures may improve the predictability of basal metabolic rate in adult subjects. Eur J Clin Nutr. 2006; **60**(12)**:** 1437-1444.

39. Morton G, Cummings D, Baskin D, Barsh G, Schwartz M. Central nervous system control of food intake and body weight. Nature 2006; **443**(7109)**:** 289-295.

40. Woods SC, Ramsay DS. Food intake, metabolism and homeostasis. Physiol Behav. 2011; **104**(1)**:** 4-7.

41. Schaumberg K, Anderson D, Anderson L, Reilly E, Gorrell S. Dietary restraint: what's the harm? A review of the relationship between dietary restraint, weight trajectory and the development of eating pathology. Clin obes. 2016; **6**(2)**:** 89-100.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

LEGENDS FOR FIGURES

Figure 1: Bland Altman plot of the differences of the means of energy intake and energy expenditure against the mean of energy intake and energy expenditure.

Figure 2: Panel A: Path diagram with standardized parameter coefficients for the direct and indirect effects of the standardised residual scores of fat mass, fat-free mass, resting metabolic rate and age (after adjusting for the influence of study differences using residuals from a linear regression model which had a term for study only), and sex on energy intake, and the squared multiple correlations (R^2) for resting metabolic rate and energy intake. The mediation model indicates that the effect of fat-free mass on energy intake was fully mediated by resting metabolic rate, while fat mass had indirect (mediated by resting metabolic rate) and direct effects on energy intake. Panel B: Path diagram with standardized parameter coefficients for the direct and indirect effects of the standardised residual scores for fat mass, fat-free mass, resting metabolic rate, cognitive restraint and age (after adjusting for the influence of study), and sex on energy intake, and the squared multiple correlations (R^2) for resting metabolic rate, cognitive restraint and energy intake. The mediation model indicates that the direct effect of fat mass was partially mediated by cognitive restraint and resting metabolic rate. FM, fat mass; FFM, fat-free mass; RMR, resting metabolic rate; DEBQ_R, restraint sub-score from the Dutch Eating Behaviour Questionnaire; EI, energy intake.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

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

	Total Sample (n = 242)		**Men (n = 114)**		**Women (n = 128)**	
	Mean ± SD	**Range (min-max)**	**Mean ± SD**	**Range (min-max)**	**Mean ± SD**	**Range (min-max)**
Age, yrs	39.7 ± 10.9	19.8-66.0	40.2 ± 10.8	20.0-64.0	39.2 ± 11.0	19.8-66.0
Stature, m	1.70 ± 0.1	1.49-2.00	1.78 ± 0.07	1.64-2.00	1.63 ± 0.06	1.49-1.79
Body Mass, kg	74.9 ± 17.3	45.5-152.4	84.0 ± 16.8	56.0-152.4	66.7 ± 13.3	45.5-128.3
BMI, kg/m^2	25.7 ± 4.9	16.7-49.3	26.4 ± 5.1	18.4-49.3	24.8 ± 4.8	16.7-47.7
Body Fat, %	27.7 ± 11.4	1.0-59.8	22.7 ± 10.9	1.0-49.4	32.2 ± 9.9	8.5-59.8

BMI, body mass index.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Table 2: Mean daily energy intake, energy expenditure, energy balance and weight change.

	Total Sample (n = 242)		**Men (n = 114)**		**Wom**
	Mean ± SD	**Range (min-max)**	**Mean ± SD**	**Range (min-max)**	**Mean**
Mean total daily energy intake, kJ/d	9761 ± 2623	5018 - 19008	11216 ± 2673	5531 - 19008	8467
Mean total daily energy expenditure, kJ/d	11011 ± 3263	5599 - 23095	13139 ± 3126	7515 - 23095	9118
Mean energy balance, kJ/d	-1250 ± 3039	-15720 - 7420	-1923 ± 3681	-15720 - 7420	-651 ±
Mean weight change, kg	-0.48 ± 0.92	-3.70 - 2.18	-0.42 ± 0.97	-3.70 - 2.10	-0.54
Resting metabolic rate, kJ/d	6497 ± 1245	4261 - 10998	7384 ± 1104	4795 - 10998	5708
PAL	1.69 ± 0.40	1.15 - 3.64	1.79 ± 0.45	1.19 - 3.64	1.60 ±

Energy balance = energy intake - energy expenditure. PAL, physical activity level (total daily energy expenditure / resting metabolic rate). Note, change in body weight measured in 229 subjects only.

Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

Table 3: Regression coefficients showing the effects of body composition, resting metabolic rate and psychometric eating behaviours on daily energy intake.

Model one (n = 242)				**Model two (n =193)**			
	B				B		
	Mean Estimate	SE	ß		Mean Estimate	SE	ß
Intercept	3909.9	1359.7		Intercept	4740.4	1668.7	
FM	-62.2	15.7	-0.29**	FM	-21.0	17.5	-0.10
FFM	33.2	27.1	0.15	FFM	45.3	27.5	0.21
RMR	0.8	0.2	0.39**	RMR	0.6	0.2	0.30*
				DEBQ_R	-760.0	188.7	-0.26**
				DEBQ_EM	161.8	208.6	0.06
				DEBQ_Ext	237.6	300.2	0.05

B, unstandardized beta coefficient; SE, standard error; β, standardized beta coefficient; FM, fat mass; FFM, fat-free mass; RMR, resting metabolic rate; DEBQ_R, restraint sub-score from the Dutch Eating Behaviour Questionnaire; DEBQ_EM, emotional eating sub-score from the Dutch Eating Behaviour Questionnaire; DEBQ_Ext, external eating sub-score from the Dutch Eating Behaviour Questionnaire. *$P \leq 0.05$, **$P \leq 0.001$. Multiple linear regression indicated that $R^2 = 0.42$ for Model one ($P < 0.001$), $R^2 = 0.48$ for Model two ($P < 0.001$). Of

Accepted manuscript

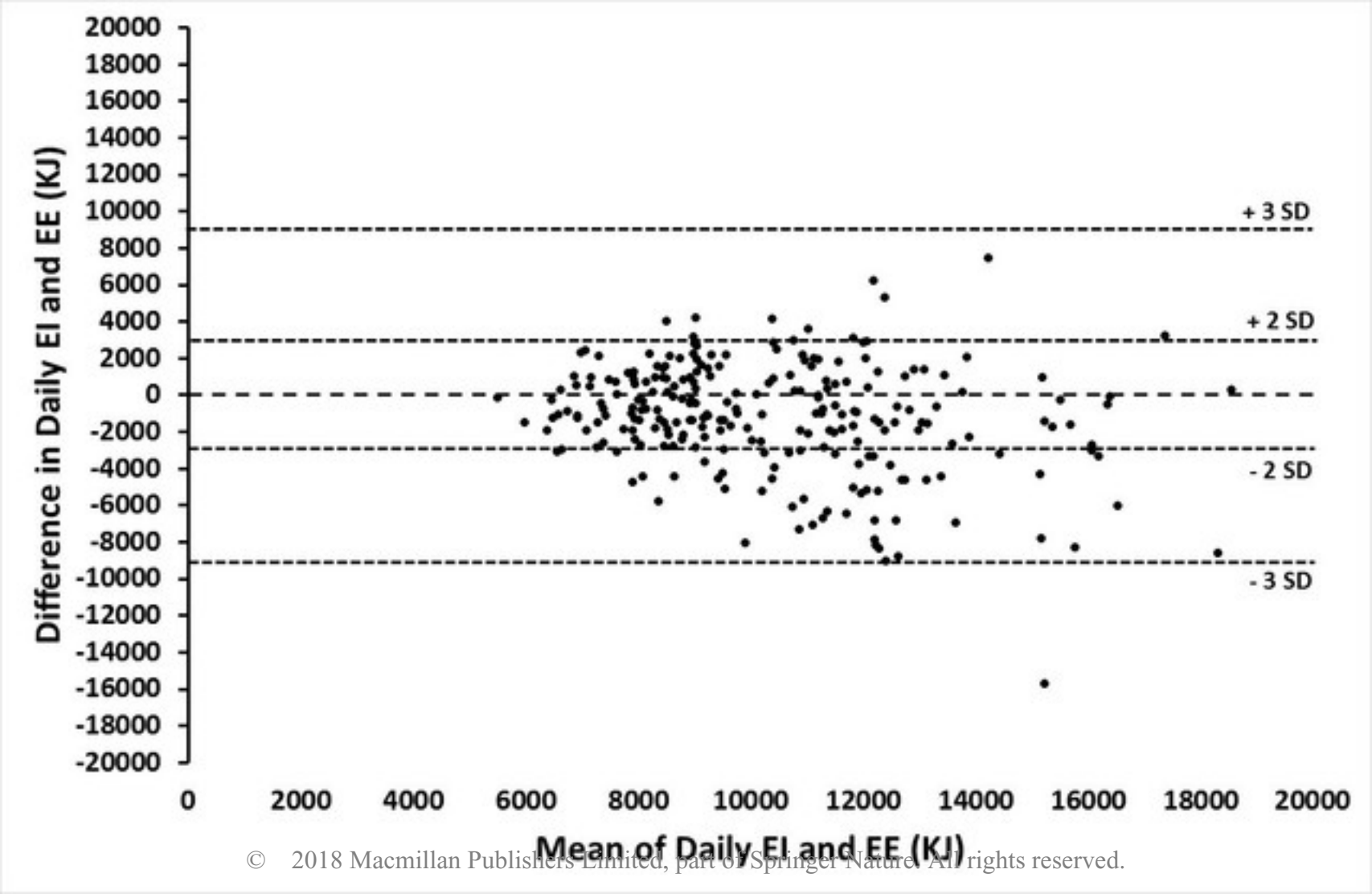
© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

AUTHOR VERSION

note, study, age and sex were also included in each model, but for clarity, regression coefficients are not reported in the table.

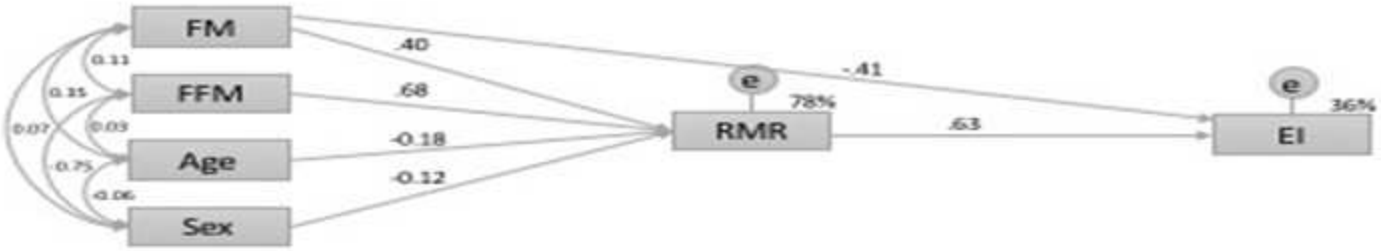
Accepted manuscript

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

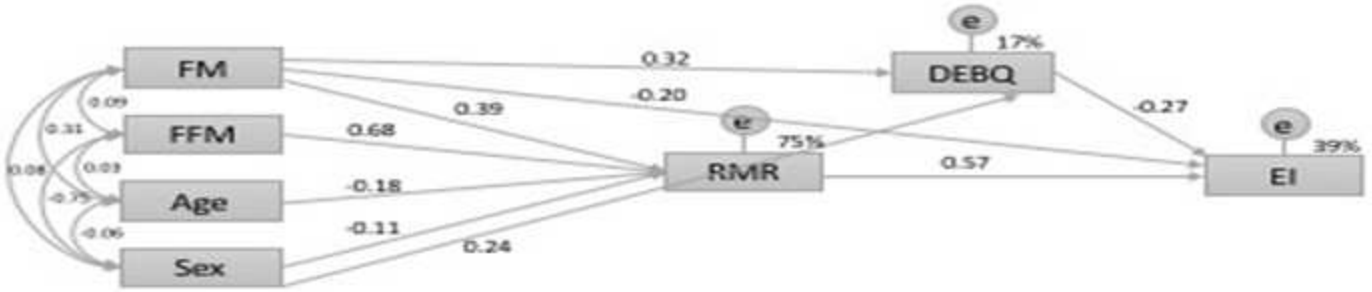


© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

A



B



© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.