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Associations between high-metabolic rate organ masses and fasting hunger: a study using whole-body magnetic resonance imaging in healthy males

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Highlights:

- Fat-free mass, skeletal muscle mass, the combined mass of high-metabolic rate organs and resting metabolic rate were associated with fasting hunger
- High-metabolic rate organ mass was more strongly associated with hunger than fat-free mass
- Within high-metabolic rate organs, the strongest individual association was between liver mass and fasting hunger
- Higher allocation of energy to the brain was associated with a lower fasting hunger
- The association between liver and hunger may reflect its role in ensuring brain's basal energy needs

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Participants of this study did not agree for their data to be shared publicly, so supporting data is not available.

Abstract

Background: Fat-free mass (FFM) has been shown to be positively associated with hunger and energy intake, an association mediated by resting metabolic rate (RMR). However, FFM comprises a heterogeneous group of tissues with distinct metabolic rates, and it remains unknown how specific high-metabolic rate organs contribute to the degree of perceived hunger.

Objective: To examine whether FFM and its anatomical components were associated with fasting hunger when assessed at the tissue-organ level.

Design: Body composition (quantitative magnetic resonance and magnetic resonance imaging), RMR and whole-body glucose oxidation (indirect calorimetry), HOMA-index as a marker of insulin sensitivity, nitrogen balance and fasting hunger (visual analogue scales) were assessed in 21 healthy males (age=25 ± 3y; BMI=23.4 ± 2.1kg/m^2) after 3 days of controlled energy balance.

Results: FFM (r_s = 0.39; p = 0.09), RMR (r_s = 0.52; p = 0.02) and skeletal muscle mass (r_s = 0.57; p = 0.04), but not fat mass (r_s = -0.01; p = 0.99), were positively associated with fasting hunger. The association between the combined mass of high-metabolic rate organs (i.e., brain, liver, kidneys and heart; r_s = 0.58; p = 0.006) and fasting hunger was stronger than with FFM as a uniform body component. The strongest individual association was between liver mass and fasting hunger (r_s = 0.51; p = 0.02). No associations were observed between glucose parameters, markers of insulin sensitivity and fasting hunger. The encephalic measure, an index of brain-to-body energy allocation, was negatively associated with fasting hunger (r_s = -0.51; p = 0.02).

Conclusions: Fasting hunger was more strongly associated with the combined mass of high-metabolic rate organs than with FFM as a uniform body component, highlighting the importance of integrating individual tissue-organ masses and their functional correlates into homeostatic models of human appetite. The association between liver mass and fasting hunger may reflect its role in ensuring the brain's basal energy needs are met.

Keywords: fat-free mass; high-metabolic rate organs; liver; energy expenditure; appetite; fasting hunger

1. Introduction

It is now established that there is a strong influence of body composition on appetite and food intake, with a number of independent studies reporting positive associations between fat-free mass (FFM) with hunger and energy intake in weight stable individuals (with little or no association with fat mass) [1-10]. These associations have been shown to be mediated by resting metabolic rate (RMR), suggesting that the physiological energetic demand arising from metabolically active tissues may influence the expression of appetite [11, 12]. It has been proposed that eating behaviours, as well other factors such as nutrient partitioning and the rate of energy expenditure, are ultimately directed towards maintaining adenosine triphosphate availability in cells (i.e., energy homeostasis) [13], but how whole-body energy expenditure influences appetite and food intake in humans remains to be fully understood.

Previous research examining the associations between FFM and appetite and EI in humans has typically used 2-compartment models of body composition (see Blundell et al. (2020) for a detailed review of literature [8]). However, the heterogeneous nature of FFM is well recognised and the individual tissue-organs that comprise FFM have distinct metabolic functions and tissue-specific metabolic rates [14-16]. Statistical models of RMR that include the contribution of individual tissue-organs explain more of the between-subject variance in RMR than 2-compartment models [14]. However, it has yet to be examined whether integrating individual tissue-organs and their mass-specific energy expenditures into homeostatic models of human appetite can improve our understanding of biological mechanisms underpinning appetite control.

Theoretical influences arising from whole-body or organ-specific substrate availability and utilisation have also been proposed to explain the effect of FFM on appetite such as the glucostatic theory of appetite [17] which postulated that glucose had a key role in appetite control. Recent studies have also proposed hepatic glucose metabolism and glycogen availability as determinants of appetite [18]. Of note, the 'selfish-brain' theory suggests that the highest metabolic priority is satisfying cerebral energy needs [19]. Considering that the brain is mostly fuelled by glucose, and is regarded as the dominant organ of appetite control [20], it could be postulated that brain glucose oxidation could exert an influence over appetite. Furthermore, while in the basal state brain glucose oxidation depends on hepatic glucose output, the liver serves as a major organ explaining the associations between FFM and RMR with hunger. However, whether the glucose demands of the brain and its supply by the liver influence hunger sensations remains to be examined.

Therefore, based on an existing study in which whole-body and tissue-organ mass and composition were measured [21], this analysis aimed to examine i) whether the mass of individual high-metabolic rate organs is associated with fasting hunger; ii) whether the mass of individual high-metabolic rate organs better explains the between-subject variance in fasting hunger as compared to assessing FFM as a single uniform body component; and iii) if whole-body or brain-specific glucose oxidation are associated with fasting hunger. It was hypothesised that the mass of high-metabolic rate organs, particularly the liver and its functional correlates (such as whole-body and brain-specific glucose oxidation), would be positively associated with fasting hunger. This would go in

agreement with the ‘selfish-brain’ theory in which cerebral energy needs being met by hepatic glucose output are a metabolic priority.

2. Materials and Methods

In the present analysis, 21 males (age = 25 ± 3y; BMI = 23.5 ± 2.2 kg/m^2; Table 1) with measures of organ mass and fasting hunger were used to investigate the associations between body composition at the tissue-organ level and fasting hunger. These data represent baseline data from a wider study in which thirty-two healthy males were recruited with the aim of assessing the physiological responses to energy balance perturbations during a 6-week subsequent overfeeding - caloric restriction - refeeding intervention [21]. This original trial was registered at clinicaltrials.gov as NCT01737034, and the present analyses were not part of the *a priori* outcomes of this study.

Participants were non-smokers, weight stable (± 2kg) during the preceding 12 months, did not use any medication, had no family history of diabetes, food allergies, contraindications for magnetic resonance imaging (MRI), and were not athletes nor following a specific diet. Data was collected between February 2010 and September 2012. The study was approved by the ethics committee of the Medical Faculty of the Christian-Albrechts University Kiel (Kiel, Germany) and conducted according to the principles of the Helsinki Declaration. All participants gave written consent after receiving oral and written information.

2.1 Study design

The intention of the present study was to examine the associations between fasting hunger and the mass of individual high-metabolic rate organs assessed via whole-body MRI. During 3 consecutive days in which participants remained in the metabolic unit of the Institute of Human Nutrition and Food Science at

Christian-Albrechts University Kiel (Kiel, Germany), measurements of fasting hunger (100-mm visual analogue scale; VAS), RMR (indirect calorimetry), body composition (quantitative magnetic resonance; QMR) and markers of insulin sensitivity (blood samples) were collected. An average of the 3 measurements of fasting hunger, RMR and body composition (QMR) was calculated and used in the present manuscript. In addition, body composition at the tissue-organ level was measured using whole-body MRI on one occasion, with skeletal muscle and adipose tissue mass measured alongside the masses of the brain, liver, kidneys and heart (i.e., high-metabolic rate organs). All data were collected after 3 days of controlled energy balance before the weight-cycling intervention started. During the 3 consecutive days in which participants were in the laboratory, total energy and protein intake were individually prescribed to each individual by nutritionists. Total energy intake was calculated as measured RMR x 1.4 to resemble a sedentary lifestyle, while protein intake was defined as 15% of total energy intake. Lastly, throughout the data collection period, the timing of the measurements conducted, as well of all the meals provided, were the same between participants.

2.2 Procedures

2.2.1 Fasting hunger

Fasting hunger was assessed using a 100-mm VAS (paper version) following an overnight fast. Participants were asked to respond to the question ‘How hungry do you feel?’ by selecting a point along the 100mm scale between the anchors ‘I am not hungry at all’ to ‘I have never been more hungry’. Each assessment was

precisely measured with a ruler to the closest 1mm. Fasting hunger was measured on 3 separate occasions during 3 consecutive days of controlled energy balance. In the present analysis, the mean of these values is present to provide a more accurate and representative value of the perception of hunger in the fasting state. The use of VAS to assess hunger perceptions has been shown to be valid and reproducible in assessing the motivation to eat, and to have a strong predictive power of subsequent energy intake [22-24].

2.2.2 Nitrogen Balance

Urinary nitrogen excretion was measured over 3 days at a group mean protein intake of 97 +/- 11g/d (for details see [21]). Twenty-four-hour urine samples were collected in 10-ml HCl 25% throughout the study and analyzed for total urea - nitrogen content by a chemiluminescence method (Chemiluminescent Nitrogen System, Model 703C, Antek Instruments, Houston, TX, USA). Measurement of nitrogen balance ordinarily has only a small error of around 1 g/d (see [25]).

2.2.3 Markers of Insulin Sensitivity

Oral glucose tolerance tests were performed in which blood samples for the determination of plasma glucose (by the glucose oxidase method (BIOSEN C-Line, EKFdiagnostic)) and insulin (by electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics)) were collected in the fasted state and at 30, 90, 120, and 180 minutes after ingestion of 75 g glucose. Area under the curve values were calculated by using the trapezoid method. An estimate of fasting insulin sensitivity was obtained by calculating the HOMA-index [26] and the Matsuda-index [27].

205

206 **2.2.4 Resting metabolic rate**

207 Measurements of VO_2 and VCO_2 were collected on 3 separate occasions during
208 3 consecutive days after an overnight fast using indirect calorimetry to calculate
209 RMR (Vmax Spectra 29n; SensorMedics; Viasys Healthcare, Bilthoven, The
210 Netherlands; software Vmax, version 12-1A; Cosmed Quark RMR, Cosmed srl,
211 Rome, Italy). Measurements were collected for 30 minutes with participants in a
212 supine position, and the first 5-10 minutes were discarded from the analyses.
213 RMR was calculated using the 5-minute steady state method [28], and data was
214 entered into the Weir equation [29].

215

216 **2.2.4.1 Whole-body, peripheral and brain-specific glucose oxidation**

217 In this study, whole-body [30], peripheral and brain-specific glucose oxidation
218 were calculated, while brain-to-body energy allocation was estimated using the
219 encephalic measure [31].

220 Resting whole-body glucose oxidation (g/min) was calculated using mean VO_2
221 and VCO_2 values during the 30-minute RMR measurement period and the
222 stoichiometric equations of Jéquier, Acheson & Schutz [30]:

223 • = 4.113 * VCO2 (L/min) – 2.907 * VO2 (L/min) – 0.375 * Protein (g/min)
224 [30]

225 Where, protein oxidation was estimated from urinary nitrogen excretion and
226 assuming 1g nitrogen = 6.25g protein.

227

Considering that in the basal state the brain oxidises glucose only, brain-specific glucose oxidation was estimated using the following equation:

- Brain Glucose Oxidation (g/min) = Brain Mass (kg) * 241kcal/kg/d / 4.1 / 24 / 60 [32]

In which 241kcal/kg/d represents the energy expenditure associated to 1kg of brain mass [33] while 4.1 represents the energetic value associated with 1g of glucose.

Peripheral glucose oxidation was assumed to equal whole-body glucose oxidation after accounting for cerebral glucose oxidation:

- Peripheral Glucose Oxidation = Whole-body Glucose Oxidation – Brain Glucose Oxidation

The ratio between brain-specific and peripheral glucose oxidation, a measure of glucose partitioning between brain and the rest of the body, was also calculated:

- Brain-Peripheral Glucose Oxidation Ratio = Brain-Specific Glucose Oxidation / Peripheral Glucose Oxidation

To examine whether brain-to-body energy allocation is influenced fasting hunger, the encephalic measure [31] was calculated using the following equation, where a higher encephalic value reflects greater energy allocated to the brain as compared to the body [34]:

- Encephalic Measure = $\frac{Brain\ Mass\ (kg)}{VO2^{1.03} * 10^{-0.06}}$ [35]

2.2.5 Quantitative magnetic resonance

Two-compartmental body composition was measured using QMR (ECHOMRI-AH; Echo Medical Systems) on 3 separate occasions during 3 consecutive days after an overnight fast. QMR uses nuclear magnetic resonance relaxometry, providing non-invasive, free of radiation, and accurate measures of body composition. Fat mass was measured in kg, and FFM was calculated by subtracting FM from total body weight.

2.2.6 Magnetic resonance imaging

Body composition assessment at the tissue-organ level was conducted using whole-body MRI, with skeletal muscle and adipose tissue mass measured alongside the masses of the following high-metabolic rate organs: brain, liver, kidneys and heart (Magnetom Avanto 1.5 T; Siemens Medical Systems). Transversal images from the wrist to ankle were obtained using a continuous axial T1-weighted gradient-echo sequence (time to repeat: 157ms; time to echo: 4ms). Regarding the brain, the protocol comprised of continuous 4mm slices with 1mm inter-slice gaps (time to repeat: 313ms; time to echo: 14ms). All the other images were obtained with an 8mm slice thickness and 2mm inter-slice gap. Assessment of the thoracic/abdominal region was obtained using breath-hold, while heart mass was assessed using the breath-navigated and pulse-triggered T2-weighted half Fourier acquisition single-shot turbo spin-echo sequence (time to repeat: 700m; time to echo: 24mm). Lastly, liver fat was determined using the 2-point Dixon method with a volume interpolated breath-hold examination.

The images collected using the MRI were manually segmented (Slice-O-Matic 4.3 software; TomoVision) by the same researcher (the intra-observer variance for repeated measurements was <2%). Total organ volume was calculated from the sum of all areas multiplied by the slice thickness (and interslice gap if applicable). Organ volume was converted to mass by multiplying the volume for its specific tissue density [36]: liver = 1.06g/cm^3, heart = 1.06g/cm^3, kidneys = 1.05g/cm^3, brain = 1.036g/cm^3, skeletal muscle = 1.04g/cm^3, adipose tissue = 0.92g/cm^3.

2.3 Statistical analyses

Data are presented as mean ± standard deviation. Data were analysed using SPSS software version 25 (IBM Corp., Armonk, New York). The Shapiro-Wilk test was used to examine for normality of distribution. Apart from subcutaneous and visceral adipose tissue, insulin and the HOMA-index, data were normally distributed. To address the skewed distribution, the same analyses were replicated with log-transformed data. As the outcomes were not altered, the raw data was presented to ease the interpretation of our findings. As not all variables were normally distributed, Spearman correlations were conducted to examine the associations between body composition (fat mass, FFM and individual organs) and RMR with fasting hunger. To account for body size and energy expenditure, the relative contribution (%) of each tissue's mass to total body weight and specific metabolic rate to RMR was also calculated (e.g., liver's mass / total body weight x 100; liver's RMR / total RMR x 100). In these analyses, the combined mass of high-metabolic rate organs represents the sum of the mass of the brain,

heart, kidneys and liver. Furthermore, residual mass was calculated by subtracting the combined mass of high-metabolic rate organs, skeletal muscle and adipose tissue from total body weight [15]. While data was initially collected from 32 individuals, only data from 21 participants were included in these analyses due to: 1) only 23 participants having measurements of organ masses using MRI and fasting hunger; 2) two participants presenting VAS fasting hunger ratings considered outliers (> 2 standard deviation below the mean). Post-hoc power calculations (G*Power v3.1) were conducted, and based on previous research examining the associations between FFM and appetite in leaner individuals (r = 0.63) [37], a statistical power of 80% and a level of significance of 5%, it was estimated that a minimum of 14 participants would be required to see a significant association between FFM (and its components) and fasting hunger. This is the first study to test the association between high-metabolic rate organs and fasting hunger. Therefore, these analyses should be considered exploratory and were not adjusted for multiple comparisons [38].

3. Results

3.1 Participant characteristics

A participant flow chart can be seen in figure 1. Descriptive characteristics, measurements of fasting hunger, RMR, whole-body composition can be observed in table 1, and markers of insulin sensitivity and glucose oxidation can be found in table 2.

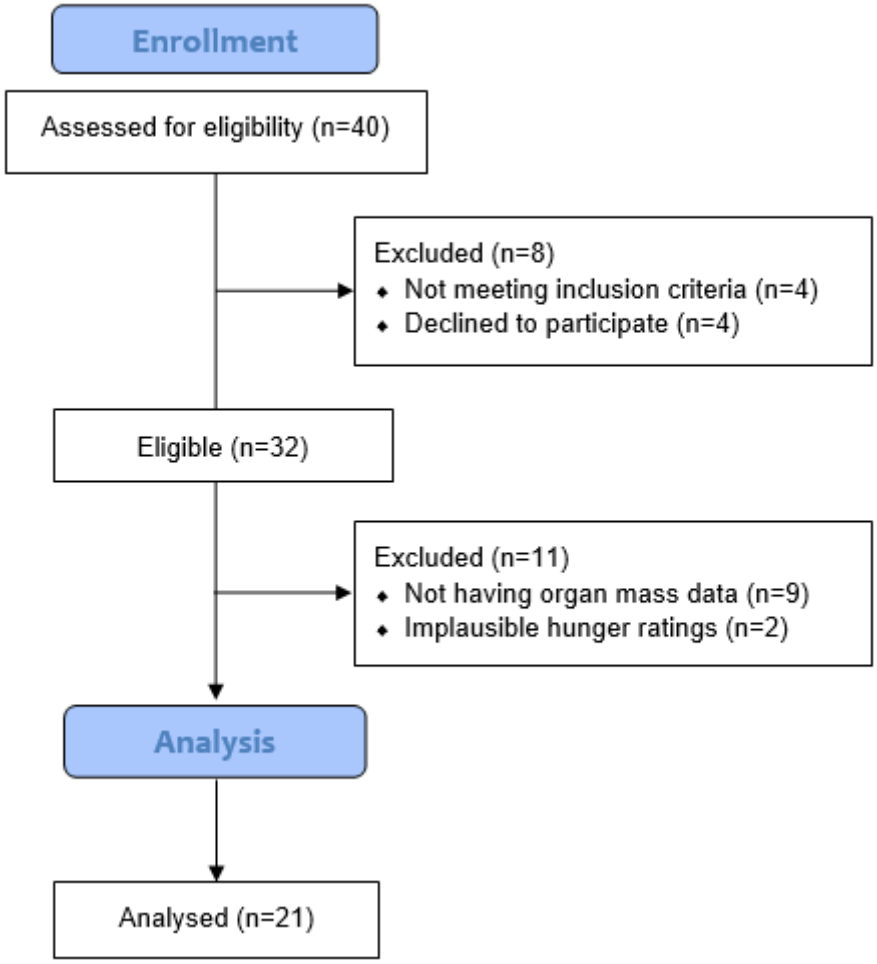


Figure 1 – Participant flow chart.

333 **Table 1 –** Descriptive characteristics, fasting hunger scores, resting metabolic
334 rate and body composition from the 21 participants.

	Mean ± SD (Range)
Age (y)	25 ± 3 (20 – 32)
Height (cm)	182.0 ± 7.3 (166.0 – 191.5)
Body weight (kg)	77.9 ± 8.8 (61.3 – 96.0)
Body Mass Index (kg/m^2)	23.5 ± 2.2 (20.7 – 29.3)
Fasting Hunger (mm)	64 ± 13 (44 – 94)
Resting Metabolic Rate (kcal/day)	1886 ± 224 (1547 – 2510)
Respiratory Quotient	0.86 ± 0.07 (0.73 – 0.96)
QMR Fat Mass (kg)	14.3 ± 5.3 (7.5 – 27.6)
QMR Body Fat (%)	18.3 ± 5.9 (9.8 – 29.3)
QMR Fat-free Mass (kg)	63.6 ± 8.1 (43.8 – 79.0)
Liver Fat (%)	6.6 ± 3.7 (4.4 – 21.3)

335 QMR, quantitative magnetic resonance.

336

337

338 **Table 2 –** Markers of insulin sensitivity and glucose oxidation from the 21
339 participants.

	Mean ± SD
Plasma insulin (mU/L)	8.98 ± 4.46
Plasma glucose (mmol/L)	4.27 ± 0.29
HOMA-index	1.68 ± 1.27
Matsuda-index	7.70 ± 3.18
Whole-body glucose oxidation (g/min)	0.13 ± 0.07
Brain glucose oxidation (g/min)	0.06 ± 0.01
Peripheral glucose oxidation (g/min)	0.08 ± 0.07
Brain-Peripheral Glucose Oxidation Ratio	0.75 ± 2.35
Encephalic measure	5.73 ± 0.67

340

341 Data regarding the assessment of body composition at the tissue-organ level, as
342 well the contribution of each tissue component to total body weight and RMR, can
343 be found in table 3. While the combined mass of high-metabolic rate organs
344 accounted for ~5% of total body weight, their contribution to RMR was ~50%.
345 Furthermore, while skeletal muscle mass represented ~39% of total body weight,
346 it only contributed to ~21% of RMR.

347

348

Table 3 – Tissue-organ masses (mean ± SD**)** and their contribution to total body weight and resting metabolic rate. For the tissue-organ masses, the range is provided in brackets.

	Mass (kg)	% Body Weight	SMR (kcal/day)	% RMR
High-Metabolic Rate Organs	3.7 ± 0.2 (3.3 - 5.1)	4.8 ± 0.4	921 ± 65	49.7 ± 3.4
Liver Mass	1.6 ± 0.2 (1.3 – 3.0)	2.1 ± 0.4	319 ± 37	17.2 ± 2.1
Fat-Free Liver Mass	1.5 ± 0.2 (1.2 – 1.9)	1.9 ± 0.2	298 ± 32	16.1 ± 2.0
Brain Mass	1.6 ± 0.1 (1.4 – 1.8)	2.0 ± 0.2	380 ± 30	20.5 ± 1.8
Kidneys Mass	0.24 ± 0.03 (0.19 – 0.34)	0.3 ± 0.04	107 ± 13	5.7 ± 0.7
Heart Mass	0.26 ± 0.05 (0.18 – 0.36)	0.3 ± 0.04	115 ± 22	6.2 ± 0.9
Skeletal Muscle Mass	30.3 ± 2.6 (27.2 – 38.2)	39.0 ± 3.5	397 ± 34	21.1 ± 1.6
Subcutaneous AT Mass	12.8 ± 3.8 (7.3 – 22.9)	16.5 ± 5.0	58 ± 17	3.4 ± 1.2
Visceral AT Mass	1.2 ± 0.8 (0.5 – 3.5)	1.5 ± 1.0	-	-
Residual Mass	31.0 ± 8.8 (14.3 – 46.3)	38.2 ± 8.5	375 ± 106	19.4 ± 4.7

AT, adipose tissue; SMR, specific metabolic rate. High-metabolic rate organs, n = 21; skeletal muscle mass, n = 14; subcutaneous and visceral adipose tissue

354 mass, n = 17; residual mass, n = 12. Specific metabolic rates were calculated
355 based on the values provided by Muller et al. [33].

356

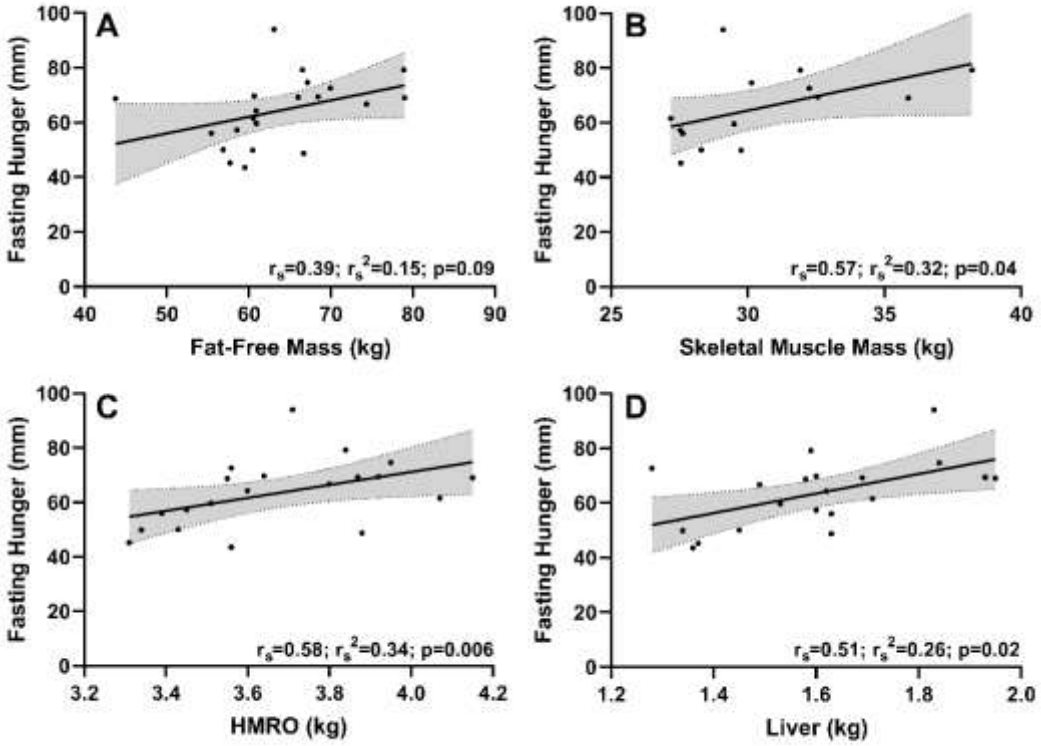
3.2 Associations Between Resting Metabolic Rate and Body Composition Components with Fasting Hunger

Associations between RMR and specific components of body composition with fasting hunger can be found in table 4. RMR and FFM, but not fat mass, were positively associated with fasting hunger, although the association with FFM was not statistically significant. Skeletal muscle mass and the combined mass of high-metabolic rate organs, and particularly the mass of the liver, were positively associated with fasting hunger. Notably, these associations presented a higher correlation coefficient (i.e., higher r_s) than FFM measured as a single uniform body component. In this sample, the mass of the brain, heart and kidneys, were not associated with fasting hunger. A visual representation of these associations can be seen in figure 2.

370 **Table 4 –** Associations between fasting hunger and resting metabolic rate or
371 body composition components.

		Fasting Hunger (mm)
RMR (kcal/day) *	r_s	**0.52**
	p	**0.02**
QMR FM (kg)	r_s	-0.01
	p	0.99
QMR FFM (kg)	r_s	0.39
	p	0.09
HMRO mass (kg) *	r_s	**0.58**
	p	**0.006**
Brain mass (kg)	r_s	0.02
	p	0.93
Kidneys mass (kg)	r_s	0.23
	p	0.32
Heart mass (kg)	r_s	0.36
	p	0.11
Liver mass (kg) *	r_s	**0.51**
	p	**0.02**
Skeletal Muscle mass (kg) *	r_s	**0.57**
	p	**0.04**

372 FFM, fat-free mass; FM, fat mass; HMRO, high-metabolic rate organs; QMR,
373 quantitative magnetic resonance; RMR, resting metabolic rate. N = 21 except for
374 skeletal muscle – n = 14. * Statistically significant association.



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376 **Figure 2 –** Scatter plots illustrating the associations between fasting hunger and
377 A) fat-free mass; B) resting metabolic rate; C) combined mass of high-metabolic
378 rate organs (HMRO); and D) liver mass. N=21 except for skeletal muscle mass
379 (n=14). Grey bands represent the 95% confidence intervals.

380

381 Liver mass was the only individual organ associated with fasting hunger, and
382 when the liver-specific energy expenditure was calculated (i.e., liver mass x 241
383 [33]), it was also found to be positively associated with RMR (r_s = 0.58; p = 0.006).
384 When the association between the combined mass of high-metabolic rate organs
385 and fasting hunger was adjusted for the liver mass (i.e., partial correlation), this
386 association became non-significant (r = 0.07; p = 0.77). No other significant
387 associations were seen between organ-specific energy expenditures and fasting
388 hunger.

389

3.3 Associations Between Whole-Body, Peripheral and Brain-Specific Glucose Oxidation and Fasting Hunger

Mean values for the markers of glucose oxidation can be found in table 2. Whole-body (r_s = -0.01; p = 0.94), brain-specific (r_s = 0.02; p = 0.93) and peripheral glucose oxidation (r_s = 0.001; p = 0.99), as well the brain-peripheral glucose oxidation ratio (r_s = 0.11; p = 0.63), were not associated with fasting hunger. However, the encephalic measure became strongly associated with fasting hunger (r_s = -0.51; p = 0.02; Figure 3). As insulin sensitivity could be a potential confounder in these relationships, partial correlations were conducted controlling for markers of insulin sensitivity (e.g., fasting plasma glucose and insulin concentrations, HOMA index and Matsuda-index), but no differences in the aforementioned associations were found.

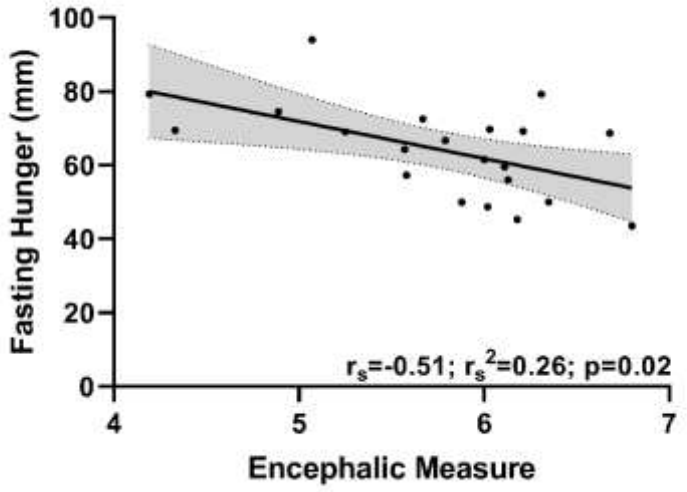

Figure 3 – Scatter plots illustrating the associations between fasting hunger and the encephalic measure. Grey bands represent the 95% confidence intervals.

4. Discussion

The aim of this analysis was to examine whether individual components of FFM were associated with fasting hunger, and whether the strength of these associations differed to that of FFM as a single uniform body component. Moreover, these analyses also explored whether whole-body, peripheral, or brain-specific glucose oxidation were associated with fasting hunger. The findings from this novel study exploring the mechanisms that influence appetite control demonstrated, for the first time, stronger associations between the combined mass of high-metabolic rate organs, and particularly the mass of the liver, with fasting hunger than with FFM as a single uniform body component. Skeletal muscle mass was also found to be positively associated with fasting hunger. Regarding glucose oxidation, whole-body, brain-specific and peripheral glucose oxidation, or the brain-peripheral glucose oxidation ratio were not associated with fasting hunger. The encephalic measure, an index of brain-to-body energy allocation, was found to be negatively associated with fasting hunger.

4.1 Associations Between Body Composition at the Tissue-Organ Level and Fasting Hunger

Previous research has demonstrated positive associations between FFM and RMR with hunger and energy intake under conditions of approximate energy balance [1, 2, 12, 39, 40]. In this study, FFM and RMR were also positively associated with fasting hunger. Interestingly, inter-individual variability in fasting hunger scores was observed (44 – 94mm). This could be attributed to several explanations, including individual biology (e.g., mass and metabolic rate of each

433 tissue), cognitive aspects (e.g., VAS interpretation) and contextual factors (e.g.,
434 timing and composition of previous meals). Nonetheless, the current findings
435 regarding the association between perceived hunger scores with fat-free mass
436 and RMR support the observations reported by previous studies [2, 7, 39].
437 Furthermore, skeletal muscle mass was positively associated with fasting hunger,
438 which goes in agreement with Cameron et al. that reported a positive association
439 between skeletal muscle and energy intake in adolescents [4]. Given its
440 contribution to RMR (~20%), this finding may corroborate the postulated 'mass-
441 dependent' effect between EE and EI, in which the energetic demand of
442 metabolically active tissue exerts influence on appetite and EI. However, a review
443 by Grannell et al. suggested that there could be specific signals arising from
444 skeletal muscle that could influence appetite [41]. A novel finding arising from this
445 study was that stronger associations (i.e., higher r_s) were observed between the
446 combined mass of high-metabolic rate organs, and particularly the mass of the
447 liver, with fasting hunger than with FFM as a single uniform body component.
448 These findings provide proof of concept that examining the associations between
449 body composition at the tissue-organ level and hunger may provide novel insight
450 into the biological signals that influence the drive to eat in humans. These findings
451 also highlight the liver as a potential critical tissue in the modulation of appetite
452 sensations, an organ that has been highlighted as a key structure influencing
453 appetite control for many decades [42].

454

455 **4.2 High-metabolic Rate Organ Mass and Fasting Hunger**

456 The finding that the associations between FFM and fasting hunger were stronger
457 when body composition was assessed at the tissue-organ level could be partially

458 explained by several reasons. In agreement with previous research [14], the
459 combined mass of high-metabolic rate organs accounted for ~50% of total RMR
460 and therefore could exert a stronger influence on fasting hunger through its
461 energetic demands. As previous studies have shown that the effects of FFM on
462 energy intake are mediated by energy expenditure (e.g., RMR and total daily
463 energy expenditure; [12, 43, 44]), and considering the greater contribution of
464 these high-metabolic rate organs to RMR, it would be plausible to suggest that
465 stronger associations might be expected between the mass of these organs and
466 hunger, as observed in the current study. Of note, liver-specific energy
467 expenditure was positively associated with RMR, which could indicate that the
468 energetic demand arising from the liver could influence the degree of perceived
469 hunger.

470 Interestingly, it should be highlighted that the correlation coefficients for the
471 associations between fasting hunger with the combined mass of high-metabolic
472 rate organs (r_s = 0.58) and the mass of the liver (r_s = 0.51) were similar. As the
473 liver was the only organ individually associated with fasting hunger, and the
474 former association became non-significant after controlling for liver mass, it is
475 possible that the association between the combined mass of high-metabolic rate
476 organs with fasting hunger was being driven by the liver.

477 It is well known that in the basal state brain glucose oxidation depends on hepatic
478 glucose output [32]. Therefore, it could be suggested that the liver may in part
479 explain the associations between FFM and RMR with hunger. Furthermore, the
480 liver has been shown to be central to the regulation of whole-body glucose, lipid
481 and amino acid metabolism in the fed and fasted states, while plasma glucose
482 concentrations are tightly controlled via hepatic glucose output to meet the brain's

483 glucose needs [32]. As the liver is therefore well placed to detect changes in
484 peripheral nutrient and energy availability, it has been implicated in a number of
485 theories of appetite [45]. For example, vagal afferent sensing of glucose in the
486 hepatic portal vein has been linked to the food intake [46], while the
487 glycogenostatic theory [47-49] focused attention on hepatic and skeletal glycogen
488 availability as a negative feedback signal in the control of food intake. However,
489 evidence that feedback from hepatic portal glucose concentrations or glycogen
490 availability provide strong negative feedback on day-to-day food intake in humans
491 is limited [50-52].

492

493 **4.3 Whole-Body, Peripheral and Brain-Specific Glucose Oxidation and**
494 **Fasting Hunger**

495 In this study, no associations were observed between whole-body, brain-specific
496 or peripheral glucose oxidation, as well as the brain-peripheral glucose oxidation
497 ratio, and fasting hunger. However, a negative association was seen between the
498 encephalic measure and fasting hunger. Given that the encephalic measure
499 reflects brain-to-body energy allocation, a higher allocation of energy to the brain
500 was associated with a lower fasting hunger in the present study. This goes in line
501 with a previous study showing that intranasal insulin increased brain energy
502 (adenosine triphosphate levels), and this neuroenergetic increase correlated with
503 a subsequent reduction in *ad libitum* buffet meal intake [53]. Furthermore, it is
504 known that insulin affects hepatic glucose production and peripheral glucose
505 utilisation to meet the brain's energy needs [32]. Therefore, it could be that fasting
506 insulin sensitivity could influence the previously mentioned associations.
507 However, controlling for insulin sensitivity, calculated using either the HOMA

index or the Matsuda-index, did not alter the strength of the correlations conducted. Of note, as these participants were healthy, young and lean, displaying no signs of insulin resistance, it is possible that these results could be different in a population in which insulin sensitivity was compromised. Moreover, the use of lean young males may have also limited the range in organ masses which could influence the outcomes from regression analyses. These findings could be interpreted to suggest that hunger is lower in individuals who prioritise the brain's energy requirements and allocate greater blood glucose to ensure these energetic needs are more readily met. This is in line with the 'selfish-brain' hypothesis which postulates that the body's highest metabolic priority is to satisfy cerebral energy needs [19], with higher hunger in those with lower brain-to-body energy allocation in the present study reflecting a central drive to increase food intake, and by extension, provision of energy to the brain. However, the cross-sectional nature of these data mean that these findings need to be replicated under conditions in which brain-to-body energy allocation is perturbed e.g., metabolic disease, starvation, significant weight loss or gain.

4.4 Limitations

The measurement of the individual high-metabolic rate organs was limited to an assessment of their mass (kg), with no direct measure of metabolic activity or energy expenditure. However, this innovative analysis has drawn attention to the relative roles of individual high-metabolic rate organs. This study employed a single measure of the expression of human appetite (fasting hunger) and did not include any objective measure of food intake or postprandial hunger profiles. Whilst a single rating may be considered a limited biomarker of appetite, it should

533 be recognised that fasting hunger is a specific feature of appetite control and
534 reflects the particular state of physiology following a period without food intake
535 (the overnight fast) and can be considered a separate biomarker from the
536 postprandial profile of hunger, hunger across the whole day (i.e., area under the
537 curve), or objective measurements of energy intake. Its validity, reproducibility
538 and predictive power regarding energy intake and feeding behaviours has been
539 demonstrated previously [22]. Lastly, the small sample size limited the statistical
540 power and prevented the use of statistical models that included several body
541 composition components. Therefore, although these findings should be
542 interpreted cautiously, they should be viewed as an initial proof of concept for
543 future research regarding the mechanisms that influence appetite control. Future
544 studies should include other markers of appetite such as objective measures of
545 food intake, subjective perceptions of hunger at different points, as well appetite-
546 related peptides, as it would allow for a more complete understanding of the
547 influence of tissue-organs on appetite.

548

549 **5. Conclusion**

550 The findings from this study demonstrated that fasting hunger was more strongly
551 associated with skeletal muscle mass and the combined mass of high-metabolic
552 rate organs, and in particular with the mass of the liver, than with FFM as a single
553 uniform body component. While the underlying metabolic or molecular
554 characteristics linking these tissues to hunger remain to be fully understood, the
555 association between liver mass and fasting hunger may reflect a major source of
556 metabolic activity, and the liver's role in ensuring the brain's basal energy needs
557 are met. Given the metabolic priority given to satisfying the brain's energy needs,

provision of energy to the brain may exert influence over hunger. Taken together, these findings suggest that including measures of body composition at the tissue-organ level in models of human appetite alongside markers of their metabolic function (e.g., structural and functional correlates) could provide novel insight into the biological mechanisms and important structure-function relationships influencing the drive to eat in humans.

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Conflict of interest:

The authors declare no conflicts of interest.

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