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Plasma LEAP2 concentration is associated with energy intake and postprandial insulin increase depending on meal size but not weight status in men

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Abstract

Aims: While LEAP2 is increasingly recognized as an appetite-regulating hormone, its role in meal regulation and the dynamics of postprandial LEAP2 concentrations remain poorly understood in humans. The aim of the study was to (1) assess postprandial LEAP2 concentrations following a recommended-energy breakfast, exploring potential association with voluntary energy intake and modulation by weight status and (2) to examine the interplay between postprandial LEAP2 and insulin concentrations.

Materials and Methods: Eighty-four men with normal weight (NW) and 48 with overweight or obesity (OW/OB) received a test meal; pre- and postprandial LEAP2 and insulin concentrations were assessed. Energy intake was calculated by multiplying the weight of the food items consumed by their energy content according to their label.

Results: Fasting LEAP2 was positively associated with glycaemia in participants with NW [Beta (95% CI): 0.289 (0.058, 0.520), $p = 0.015$] but not with OW/OB. Sixty-seven participants consumed the entire test meal (CMC, complete meal consumption); the rest consumed only part (PMC, partial meal consumption). Postprandial LEAP2 concentration was higher in the PMC ($p = 0.017$) than in the CMC group and in participants with OW/OB ($p = 0.046$). Pre ($p = 0.027$) and postprandial ($p = 0.031$) LEAP2 was inversely related to ingested calories, but only in PMC and independent of weight status. Postprandial insulin increase ($p < 0.001$) depended on LEAP2 only in CMC ($p = 0.017$) and was independent of weight status.

Conclusions: The relationship of LEAP2 with energy intake and postprandial insulin increase is influenced by meal size but not weight status.

KEYWORDS
energy intake, insulin, LEAP2, meal size

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1 | INTRODUCTION

Liver-enriched antimicrobial peptide 2 (LEAP2) was identified in 2018 as an endogenous inhibitor of the growth hormone secretagogue receptor (GHSR), which binds the hormone ghrelin.[1–3] Circulating LEAP2 acts in opposition to ghrelin, decreasing during energy deficits and increasing after refeeding in mice[1,4] and in humans.[5] Conversely, fasting plasma LEAP2 concentrations are higher in individuals with obesity (OB) compared to normal-weight subjects,[6–8] consistent with findings in mice.[1,6] Positive correlations between LEAP2, body mass index (BMI), adiposity measures such as waist circumference and glycaemia have been documented in groups with normal weight (NW), overweight (OW) or OB.[6,7] The mechanisms underlying fluctuations in plasma LEAP2 levels are still being elucidated.[9] Preclinical studies indicate that LEAP2 secretion from hepatocytes—presumably the primary source of circulating LEAP2—is upregulated by insulin and glucose, suggesting that glucose homeostasis and its regulatory pathways play a key role in modulating LEAP2 concentrations in humans.[10,11] However, comprehensive human studies specifically examining plasma LEAP2 responses under various pre- and postprandial conditions, or comparing levels during different phases of obesity, are currently lacking.

While LEAP2 is increasingly recognized as an appetite-regulating hormone, its role in meal regulation and the dynamics of postprandial LEAP2 concentrations remain poorly understood in humans. Few studies have examined plasma LEAP2 levels following standardized solid or liquid breakfasts, with some reporting no statistically significant changes[6,7,12] and others noting a postprandial increase, particularly in females with OW/OB.[6,7] LEAP2 levels have also been measured after ad libitum intake of high-energy lunches,[13,14] yet limited research explores LEAP2 responses in natural conditions, such as after a breakfast formulated to meet energy recommendations,[15] and whether these responses vary by weight status. Additionally, LEAP2's potential role in postprandial glucose metabolism is scarcely examined. LEAP2 infusion in lean men reduced postprandial glucose excursions without affecting insulin,[14] but whether endogenous LEAP2 regulates postprandial insulin remains unknown. It is also unclear if these postprandial changes in LEAP2 are meal-size-dependent. Previous studies have shown that both exogenous[14] and endogenous[8] LEAP2 are inversely associated with ad libitum energy intake, yet whether postprandial LEAP2 concentration depends on energy intake remains to be determined.

Here, we hypothesized that LEAP2 postprandial response depends on meal size and could be modulated by weight status. This study assessed postprandial LEAP2 concentrations following a recommended-energy breakfast and explored the potential modulation by weight status and the association with energy intake. Furthermore, we examined the interplay between postprandial LEAP2 and insulin levels. We analysed data and samples from a pre-existing cohort with a broad range of BMI values, collected from a study we previously described.[8]

2 | METHODS

2.1 | Study participants

Data and plasma samples for LEAP2 assessment originate from a study conducted at Uppsala University that recruited healthy volunteers with normal eating habits between 08/2011 and 08/2018 to study genetic and epigenetic effects of food intake, as previously described[8,16] (Figure S1). Only data and plasma samples from male participants with pre-and postprandial LEAP2 and insulin measurements were included in this study ($n = 132$). Female participants were excluded due to their small sample size and the uneven distribution across weight status categories for insulin measurements. Ethical approval was granted by the ethical review board (Uppsala). Informed consents were obtained. The study was carried out following the Declaration of Helsinki and registered in clinicaltrials.gov (NCT01863212).

2.2 | Study protocol

Figure 1 depicts the study design.

Participants arrived at the Institute in the morning after a 12-h fast and underwent an initial evaluation, including medical and family history. Weight, height and waist circumference were measured using standard procedures.[17] Participants were categorized into NW (BMI 18.5–24.99 kg/m^2) and overweight or obesity (OW/OB; BMI ≥ 25 kg/m^2).[18] Next, a blood sample was obtained by venipuncture. Participants received a breakfast test meal consisting of one piece of whole-grain bread (70 g, Fruktkuse, Coop Sverige AB) with 3 slices of Leerdammer cheese (60 g) and 250 g of Quark curd cheese (Arla, Sweden). The meal comprised 583.13 kcal, of which 205.36 kcal (35.33 kcal%) was protein, 182.79 kcal (31.33 kcal%) were total carbohydrates, and 194.98 kcal (33.33 kcal%) was fat. Water (30 mL) was provided as a beverage. The caloric content was estimated to cover 20% of total daily energy expenditure for men.[19] Participants were instructed to consume as much of the test meal as they wanted within 15 min. When available, the rest of each food item was weighed, and energy intake was calculated by multiplying the weight of the food items consumed by their energy content according to their label. Fullness sensation after test meal consumption was estimated using a 100-mm visual analogue scale (VAS).[20] A second blood sample was taken 120 min after breakfast was finished. Plasma samples were obtained by centrifugation (1300 rcf) and stored at −80°C until analysis.

2.3 | Biochemical analyses

Glycaemia was measured using an Accu-Chek Aviva blood glucose meter (Roche Diagnostics, USA). LEAP2 was assessed using an immunoassay from Phoenix Pharmaceutical, USA (EK-075-40, intra- and inter-assay variations of 6.4 and 9.9%, respectively, validated in

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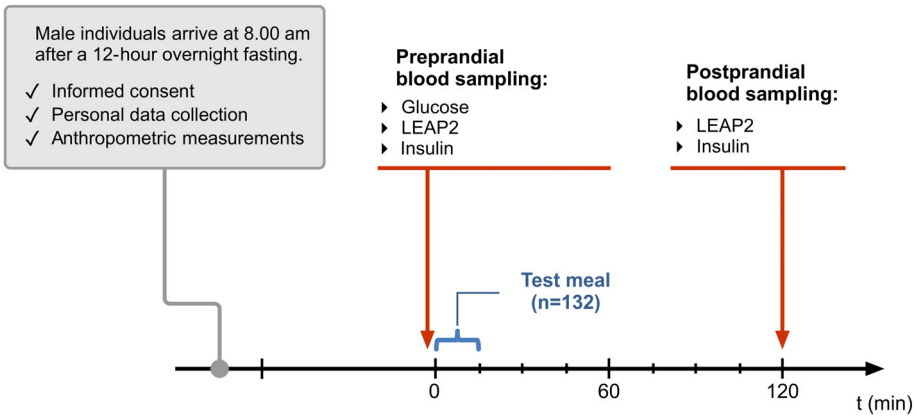

FIGURE 1 Overview of study design. HC, high calorie; LC, low calorie; LEAP2, liver-enriched antimicrobial peptide 2.

TABLE 1 Baseline characteristics of the participants.

	All ($n = 132$)	NW ($n = 84$)	OW/OB ($n = 48$)	*p*-value
BMI (kg/m^2)[a]	23.9 (18.7–36.8)	22.5 (18.7–24.8)	26.4 (25.0–36.8)	**<0.001**
Age (years)[b]	26 (24–28)	26.0 (23.0–27.7)	26.5 (25–28)	0.578
LEAP2 (ng/mL)[c]	12.93 (12.06, 13.87)	12.27 (11.22, 13.42)	14.18 (12.68, 15.86)	**0.045**
Glycaemia (mmol/L)[d]	5.11 (0.46)	5.08 (0.45)	5.15 (0.48)	0.402
Insulin (μU/mL)[d]	6.06 (1.83)	5.75 (1.72)	6.92 (1.74)	0.053
HOMA index[d]	1.39 (0.43)	1.34 (0.43)	1.47 (0.44)	0.375

Note: Values in bold represent statistical significance using the Student's *t*-test or Mann–Whitney *U* test.
Abbreviations: HOMA, homeostatic model assessment; NW, normal weight, OW/OB, overweight/obesity.
[a]Data are presented as median (range).
[b]Data are presented as median (IQR).
[c]Data analysis performed on natural log-transformed values. Back-transformed values are presented as geometric means and 95% confidence intervals.
[d]Data are presented as mean and standard deviation (SD).

Reference 6). Assessment was not influenced by the storage time of the samples (data not shown). Insulin was assessed using a noncompetitive immunometric assay (12 017 547 122; Roche Diagnostics, USA). Insulin resistance was estimated by the homeostasis model assessment for insulin resistance (HOMA index).[21] Assessment of plasma levels of other peptide hormones (acyl ghrelin, GLP-1 and PYY) was not performed because sample collection did not include recommended protocols to guarantee their stability (e.g., protease inhibitors).

2.4 | Statistics and data analyses

Statistical analyses were performed using R software version 4.0.3 or SPSS Version 28.0. Variables normally distributed were summarized as mean ± standard deviation (SD); non-normally distributed data were presented as median (interquartile range, IQR), and categorical data were summarized as frequency counts and percentages. Age and BMI were presented as median (range). LEAP2 concentration was log-transformed prior to analysis to account for positive skewness. Multivariable linear regression, binary logistic regression and linear mixed-effects models were used to test the relationship of LEAP2 concentrations with different outcome variables. Interaction analyses were performed to estimate if BMI modified the association between LEAP2 and the variables assessed. Power calculations were omitted as the data were derived from a pre-existing clinical trial.[8] Further details can be found in the Supplementary Methods.

3 | RESULTS

3.1 | Characteristics of the participants and meal consumption

The study included 132 males with a mean age (range) of 26 (20–40) years. Based on BMI, 84 participants were categorized as with NW, and 48 as participants with OW/OB. Table 1 details participant characteristics. Preprandial LEAP2 concentration was higher in participants with OW/OB. Conversely, glycaemia, insulin and HOMA index did not differ between individuals with NW or OW/OB.

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TABLE 2 Associations between pre-prandial LEAP2 concentration and anthropometric and metabolic measures.

Plasma LEAP2 concentration (ng/mL)	All [b] Beta (95% CI)	*p*	BMI × LEAP2 interaction *p*	NW [c] Beta (95% CI)	*p*	OW/OB [c] Beta (95% CI)	*p*
BMI (kg/m^2)[a]	1.746 (0.263, 2.485)	**0.002**	-	−0.077 (−0.785, 0.632)	0.831	1.577 (0.163, 2.990)	**0.029**
Waist circumference (cm)[a]	6.153 (1.569, 8.517)	**0.001**	-	−0.173 (−2.692, 2.345)	0.892	6.230 (1.057, 11.403)	**0.019**
Glycaemia (mmol/L)	0.186 (0.009, 0.362)	**0.039**	**0.017**	0.289 (0.058, 0.520)	**0.015**	0.091 (−0.186, 0.368)	0.514
Insulin (uU/ml)	0.17 (−0.04, 0.37)	0.107	0.619				
HOMA index	0.32 (0.05, 0.59)	**0.019**	0.677				

Note: Data are presented as the regression coefficient beta (95% confidence interval) from linear regression analyses.
Abbreviations: BMI, body mass index; NW, normal weight; OW/OB, overweight/obesity.
[a]Not adjusted for BMI.
[b]Adjusted for age and BMI.
[c]Adjusted for age.

TABLE 3 Characteristics of meal consumption groups.

	CMC ($n = 67$)	PMC ($n = 65$)	*p*-value
Participants with NW[a]	43 (64)	41 (63)	0.895
Participants with OW/OB[a]	24 (36)	24 (37)	
Energy intake (kcal)[b]			
All	583 (11)	476 (63)	**<0.001**
NW		485 (60)	0.117
OW/OB		459 (67)	
Fullness (cm)[c]	7 (3; 10)	7 (2; 9)	**0.025**
Protein intake[c]			
kcal	205 (202; 206)	144 (111; 172)	**<0.001**
kcal%	35.33 (35.16; 35.68)	29.90 (25.42; 34.23)	**<0.001**
Carbohydrate intake[c]			
kcal	183 (179; 184)	158 (149; 173)	**<0.001**
kcal%	31.33 (31.26; 31.40)	33.14 (32.19; 34.36)	**<0.001**
Fat intake[c]			
kcal	195 (188; 197)	180 (174; 185)	**<0.001**
kcal%	33.33 (33.00; 33.47)	36.89 (33.56; 40.39)	**<0.001**
Binary logistic regression[d]			
Preprandial LEAP2 (ng/mL)	0.651 (0.447)		0.145
BMI (kg/m^2)	0.055 (0.061)		0.363

Note: *p*-value testing for differences in participant characteristics by meal consumption group using the Student's *t*-test or Mann–Whitney *U* test for quantitative variables and Fisher exact test for qualitative data.
Abbreviations: CMC, complete meal consumption; PMC, partial meal consumption.
[a]Frequency (%).
[b]Mean (SD).
[c]Median (IQR).
[d]Data are presented as the regression coefficient beta (standard error).

3.2 | Preprandial LEAP2 concentration is directly associated with glycaemia and HOMA index

Preprandial plasma LEAP2 concentration exhibited a direct relationship with glycaemia and HOMA index, and no associations with insulin (Table 2). Statistically significant interactions between BMI and pre-prandial plasma LEAP2 concentrations were observed for glycaemia ($p = 0.017$), and when analysing participants with NW and OW/OB separately, pre-prandial LEAP2 concentration displayed a positive relationship with glycaemia in participants with NW ($p = 0.015$) but not with OW/OB ($p = 0.514$). Preprandial LEAP2 concentration exhibited a direct relationship with BMI and waist circumference only in participants with OW/OB.

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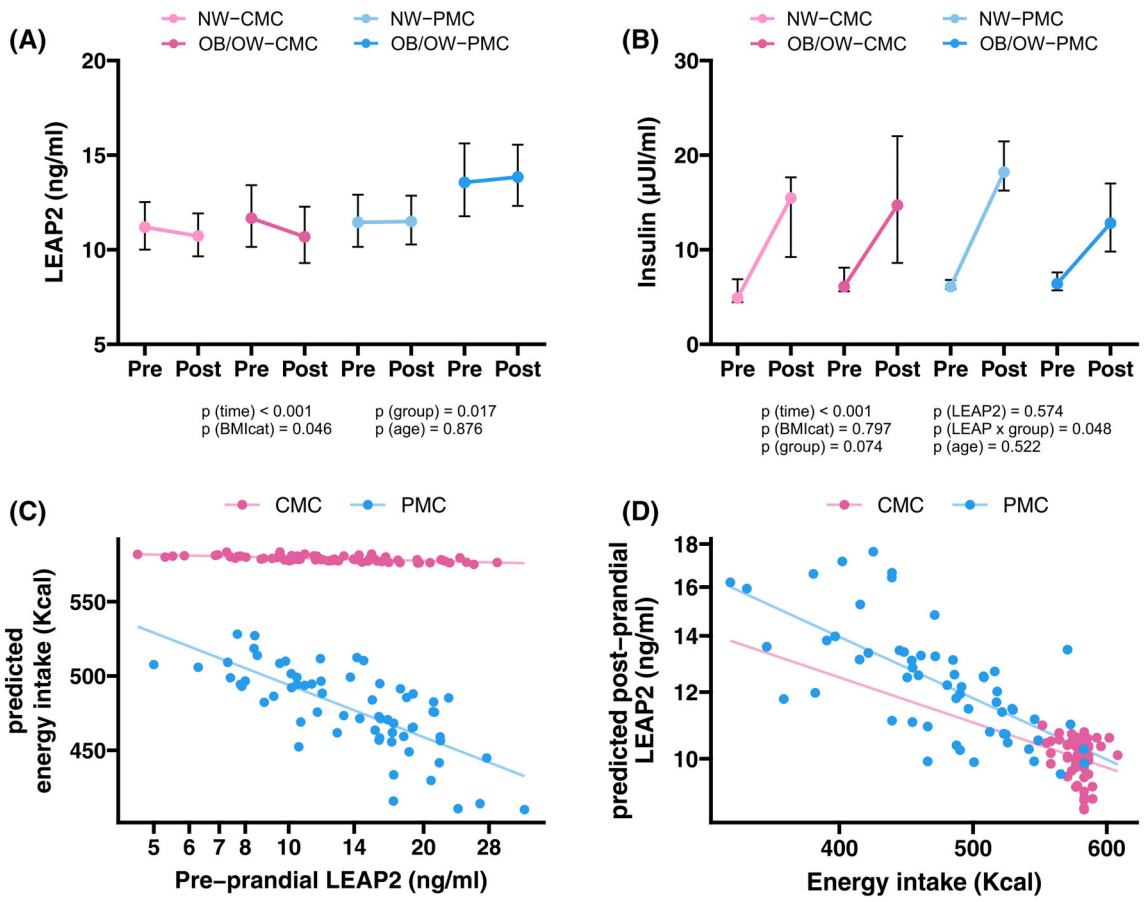

FIGURE 2 (A, B) Pre- and postprandial LEAP2 (A) and insulin (B) concentrations according to group (CMC or PMC) and BMI category (NW or OW/OB). Data were analysed using linear mixed-effects models controlling for time (pre- vs. postprandial), age and BMI category (A), and including LEAP2 concentration in the model (B). Coloured points and lines represent the geometric mean and 95% CI (A) or the median and IQR (B). *p* values for main effects and for the interaction between LEAP2 and group (B) are shown. (C, D) Scatterplots showing values predicted from multiple linear regression models for energy intake versus preprandial LEAP2 (C) and for postprandial LEAP2 versus energy intake (D), according to group (CMC or PMC). CMC, complete meal consumption; NW, normal weight; OW/OB, overweight/obesity; Post, postprandial; PMC, partial meal consumption; Pre, preprandial.

3.3 | Meal consumption

Based on the test meal consumption, we identified 2 groups of participants (Table 3). One group consumed the entire test meal offered, constituting the 'complete meal consumption' (CMC) group. Another group consumed only part of the test meal, forming the 'partial meal consumption' (PMC) group. A similar number of individuals with NW or OW/OB were identified in each group. Energy intake was higher in the CMC than in the PMC group. In the latter, no statistical differences were observed between participants with NW or with OW/OB. In line with energy intake, fullness sensation was higher in the CMC group than in the PMC group. The nutrient composition of the meal consumed by the PMC group showed a lower percentage of protein intake, accompanied by a higher proportion of fat and carbohydrates. Binary logistic regression analysis revealed that neither pre-prandial LEAP2 concentration nor BMI were associated with CMC.

3.4 | Postprandial decrease in LEAP2 concentration depends on meal size and is associated with insulin increase

Figure 2A shows pre-prandial and postprandial concentrations of plasma LEAP2 according to BMI category and meal consumption group (CMC or PMC). Linear mixed-effects models showed a significant postprandial decrease in plasma LEAP2 concentration (effect of time, $p < 0.001$), as previously reported.[8] Postprandial LEAP2 concentrations were 1.2 fold higher in the PMC group compared to the CMC group (PMC: 12.37 [11.29, 13.55] ng/mL; CMC: 10.21 [9.25, 11.26] ng/mL, effect of group, $p = 0.017$), as well as in participants with OW/OB compared to NW (OW/OB: 12.27 [10.95, 13.74] ng/mL; NW: 10.70 [9.83, 11.67] ng/mL, effect of BMI category, $p = 0.046$), and no significant interaction between BMI category and group was observed ($p = 0.136$) (Figure 2A). Figure 2B depicts preprandial and postprandial plasma insulin concentrations according to BMI category

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and group. Linear mixed-effects models showed a significant increase in postprandial plasma insulin concentration (effect of time, $p < 0.001$), with no effect of group ($p = 0.074$), BMI category (0.797) or LEAP2 concentration ($p = 0.574$). However, a significant interaction between LEAP2 concentration and meal consumption group was observed ($p = 0.048$). When analysing both groups separately, linear mixed-effects models showed that the postprandial insulin increase (effect of time $p < 0.001$) depended on LEAP2 only in the CMC group ($p = 0.017$), with no BMI category ($p = 0.138$) effect.

3.5 | Pre-and postprandial LEAP2 concentrations are inversely associated with energy intake in PMC, but not in the CMC group

Multivariable regression analysis revealed that pre-prandial LEAP2 concentration displayed an inverse relationship with total ingested calories in the PMC group (Beta [95% CI]: −41.51 [−78.17, −4.85], $p = 0.027$) but not in the CMC group (Beta [95% CI]: −2.85 [−8.25, 2.54], $p = 0.293$) (adjusting for age and BMI, Figure 2C). No significant interactions between plasma LEAP2 concentration and BMI were observed ($p = 0.846$ for PMC and 0.440 for CMC). No significant associations were found between pre-prandial LEAP2 concentration and individual macronutrient intake (data not shown). Post-prandial LEAP2 concentration also displayed an inverse relationship with ingested calories in the PMC group [Beta (95% CI): −0.002 (−0.003, −0.000), $p = 0.031$] but not in the CMC group (Beta [95% CI]: 0.000 [−0.009, 0.009], $p = 0.964$) (adjusting for age and BMI, Figure 2D). No significant interactions between energy intake and BMI on postprandial LEAP2 concentration were observed ($p = 0.577$ for PMC and 0.561 for CMC).

4 | DISCUSSION

Our findings indicate that LEAP2 concentrations are modulated by meal size, with higher levels observed when the available meal is only partially consumed compared to complete consumption. Energy intake was inversely associated with both pre- and postprandial LEAP2 levels in individuals who partially consumed the meal. In contrast, postprandial LEAP2 levels were associated with the postprandial insulin response among those who completely consumed the meal. Notably, these effects were independent of the participants' weight status.

Ad libitum feeding is the usual daily eating pattern in modern societies. In this study, participants were offered a standardized breakfast test meal designed according to energy intake recommendations. While the composition of the meal was predefined and standardized, participants could freely choose the quantity and combination of food items (bread, cheese or curd), thereby ensuring voluntary food intake. This design aimed to replicate natural breakfast conditions, enabling the assessment of eating behaviours, appetite regulation and energy intake in a setting that closely resembles real-life consumption patterns. The breakfast test meal was designed to provide between 15% and 25% of the subjects' total daily energy expenditure, aligning with the recommended breakfast energy intake.[15] Nearly half of the subjects did not consume the entire meal, with the energy consumed covering an average of 17% of their daily energy requirement. This pattern was observed regardless of the BMI. Notably, this finding aligns with data from the European Prospective Investigation into Cancer and Nutrition (EPIC) study, which reported that breakfast typically accounts for 17% of daily energy intake in Sweden, equivalent to approximately 300–500 kcal.[22] In this context, the 583 kcals provided by our test breakfast exceeded the average reported intake and may have been perceived as excessive by some participants. This outcome was likely influenced by a combination of physiological, psychological and environmental factors. Despite the reported anorexigenic effects of LEAP2, our findings indicate that pre-prandial LEAP2 concentration did not predict CMC across participants, suggesting a potential ceiling effect of the anorexigenic action of LEAP2 (see below). Nevertheless, we cannot rule out the possible involvement of other appetite-regulating hormones that were not assessed in this study. Peptide hormones glucagon-like peptide-1 (GLP-1), peptide YY (PYY) and cholecystokinin (CCK) are well-established satiety signals known to reduce subsequent meal size and energy intake.[23,24] Given their established roles in appetite regulation, these hormones may have influenced participants' decisions to stop eating before finishing their meals, independently of LEAP2 levels.

Few studies have assessed the regulating role of LEAP2 on food intake in humans. Intravenous LEAP2 administration has been shown to suppress food intake in healthy men.[14] Endogenous LEAP2 also seems to affect appetite according to interventional studies including adult men and women with NW and with OW/OB, which show that pre-prandial plasma LEAP2 concentration is inversely associated with hunger sensation[7] and subsequent food intake.[8] LEAP2 was higher in subjects with OW/OB, in line with other studies,[6–8] indicating that, unlike the relationship with energy intake, LEAP2 concentration may depend on the weight status of the subject. However, elevated LEAP2 levels do not necessarily reflect enhanced LEAP2 activity. Instead, they may represent a compensatory upregulation in response to reduced sensitivity to LEAP2, indicating a potential 'LEAP2-resistant' state. This interpretation is supported by preclinical studies showing that obese mice become unresponsive to the anorexigenic effects of LEAP2 administration.[25,26] These findings mirror the well-established concept of leptin resistance in obesity, where elevated hormone levels fail to elicit their expected physiological effects.[27] The specific metabolic conditions under which LEAP2 retains or loses efficacy in obesity remain to be fully elucidated. Our current results also showed that pre-prandial LEAP2 concentrations were inversely associated with energy intake in the PMC, but not in the CMC group, independently of BMI. This suggests that the inverse relationship between fasting LEAP2 levels, an anorectic signal and subsequent energy intake occurs only below a certain energy level and is not influenced by the weight status of the subject. The mechanism underlying the lack of association between pre-prandial LEAP2 concentration and energy intake in subjects who consumed the entire test meal in our study remains unknown. As discussed above, it is possible that other

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neuroendocrine or metabolic mechanisms or even external factors regulating food intake could override the anorectic effects of LEAP2. In any case, our results suggest that LEAP2 influences energy intake only below a certain energy threshold. Given that LEAP2 is primarily secreted via a constitutive pathway, its circulating levels are likely to reflect metabolic status over longer time scales rather than respond acutely to individual meals. This suggests that LEAP2 may be more involved in the maintenance of long-term energy homeostasis than in the immediate, meal-to-meal regulation of food intake.

In addition, our study provides novel insight into the implications of postprandial plasma LEAP2 concentration. First, we found that postprandial LEAP2 concentrations were inversely associated with energy intake only in subjects who did not completely consume the meal, suggesting that energy intake regulates LEAP2 production only within certain energy levels. This observation aligns with previous studies showing that postprandial LEAP2 concentration is associated with reductions in appetite.[13] In contrast, postprandial LEAP2 concentrations were no longer associated with energy intake in subjects who consumed the entire meal, suggesting that the effect of energy intake on LEAP2 becomes negligible once a specific meal size or percentage of daily energy requirements is reached. Physiologically, this could reflect a ceiling effect, where once a substantial caloric load is ingested, potentially fulfilling acute metabolic demands or surpassing a threshold related to nutrient sensing, additional intake does not further affect LEAP2 levels. It is also possible that other post-ingestive signals, such as insulin, gut-derived hormones or hepatic nutrient sensing pathways, become dominant in regulating LEAP2 secretion, thereby diminishing the apparent association with meal size in these individuals. Secondly, we observed that postprandial LEAP2 concentration was higher in the PMC than the CMC group. Consequently, the postprandial decline in LEAP2 levels was attenuated in the partial meal consumers. The key factors regulating postprandial plasma LEAP2 concentrations in humans remain somewhat unclear and are still a matter of debate. Current evidence suggests that LEAP2 levels are modulated not only by energy intake but also by specific metabolites and the nutrient composition of meals. Several studies have shown that plasma LEAP2 concentrations decrease following β-hydroxybutyrate administration in both mice[28] and healthy individuals.[5] However, findings remain inconclusive, as an increase in plasma LEAP2 was observed after the ingestion of a ketone monoester drink, but not after an isovolumetric glucose drink.[29] Additional clinical studies support the notion that both circulating metabolites and macronutrient composition significantly influence LEAP2 regulation. For instance, carbohydrate-rich test meals—as well as oral glucose or lactate administration—have been shown to elevate plasma LEAP2 levels,[5,13] whereas high protein intake appears to suppress them.[8,30] Therefore, it seems likely that the smaller postprandial decrease of LEAP2 observed in the PMC group was due to a weaker inhibitory effect of protein intake in this group. The significance of the differing changes in plasma LEAP2 levels between groups remains unclear, as these variations do not appear to be linked to fullness sensation, which was lower in the PMC group compared to the CMC group and not associated with postprandial LEAP2 in our study (data not shown). Postprandial changes in LEAP2 may be influenced by a complex interplay between energy intake, macronutrient composition, the proportion of energy requirements met and the weight status of the subjects. These results highlight the specific role of LEAP2 in regulating feeding in humans.

LEAP2 has been proposed to play a role in glucose homeostasis in subjects with NW and OWOB[14,31]; however, the interplay between postprandial LEAP2 and insulin remains scarcely studied.[14] The observed positive associations between pre-prandial LEAP2 concentrations and both glycaemia and insulin resistance (as indicated by the HOMA index) suggest that LEAP2 is involved in glucose metabolism, particularly in regulating glycaemia in anticipation of food intake. Nonetheless, we cannot exclude the possibility that the role of LEAP2 is influenced by complex interactions with other glucose-regulating hormones, such as GLP-1. This potential interplay remains largely unexplored and was not assessed in the present study. Although the association between LEAP2 and insulin resistance was independent of BMI, the regulation of glycaemia was observed only in subjects with NW, indicating that LEAP2 may primarily contribute to glucose regulation in subjects without obesity. Additionally, LEAP2 regulated the postprandial insulin response only when a complete meal was consumed and independently of the weight status of the subjects. Previous studies revealed an insulinotropic effect of LEAP2 infusion during fasting, but not following a standardized liquid mixed meal.[14] Our findings suggest that the postprandial interaction between these hormones occurs above a certain energy threshold, beyond which postprandial LEAP2 concentrations are unaffected by the amount of energy ingested.

The strengths of the present study include a larger sample size compared to previous studies[6,7,13] and the adjustment of analyses for potential confounding factors. The high stability of LEAP2 in plasma, likely attributable to its chemical structure featuring two disulphide bonds, a β-hairpin and a 3_{10}-helix,[32] allowed for the analysis of plasma samples after extended storage periods. However, several limitations influence the conclusions that can be drawn from this study. First, we only included male participants from the original study.[8] Also, lasma concentrations of other peptide hormones were not measured, and we could only take a single postprandial blood sample. Similarly, the absence of postprandial glucose measurements limited our ability to interpret the relationship between postprandial LEAP2 and insulin. Our study did not specifically recruit participants with obesity; therefore, the OW/OB group was composed mainly of participants with BMI < 33 kg/m^2. Due to the limited number of participants with BMI >30 kg/m^2, individuals with BMI >25 kg/m^2 were grouped into a single overweight/obese (OW/OB) category. This approach may mask important metabolic differences between overweight and obese individuals, as BMI alone does not fully capture variability in metabolic health. Since our study included men of mainly northern European origin, our findings might not be generalizable to other ethnic groups. Finally, it is worth mentioning that the specific composition of the test meal used in our study may have shaped the observed postprandial LEAP2 dynamics since previous studies have shown divergent LEAP2 responses to carbohydrate-, protein- and ketone-rich meals.[5,8,13,29,30]

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In conclusion, our study sheds light on the association between LEAP2 and energy intake. Our findings emphasize the significance of meal size in LEAP2's physiological role, underscoring the specific roles of this hormone for human food intake. These insights highlight the potential of LEAP2 as a target for developing interventions to improve feeding behaviour and address eating disorders and obesity.

AUTHOR CONTRIBUTIONS

Funding acquisition, supervision, reviewing and editing, overall responsibility for the project: HBS. Drafting the manuscript: MFA. Methodology, formal analysis, investigation, resources and data curation: MFA, PNDF, OET and MP. All authors approved the submitted and published versions.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data described in the manuscript, code book and analytic code will be made available upon request.

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