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LEAP2 is associated with impulsivity and reward sensitivity depending on the nutritional status and decreases with protein intake in humans

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Abstract

Aim: Liver-expressed antimicrobial peptide 2 (LEAP2) dynamics in human plasma and its association with feeding behaviour remain poorly understood. Therefore, this study aims: (a) to investigate fasting LEAP2 in participants with normal weight or with overweight or mild obesity (OW/OB); (b) to study the association between fasting LEAP2 and anthropometric and metabolic traits, feeding behaviour, *LEAP2* genetic variants and blood cell DNA methylation status; and (c) to ascertain postprandial changes in LEAP2 after high protein intake and the association with feeding behaviour and food intake.

Methods: Anthropometric and behavioural measures, genotyping, methylation profiling, plasma glucose and LEAP2 concentrations were assessed in 327 females and males. A subgroup of 123 participants received an *ad libitum* high-protein meal, and postprandial LEAP2 concentration and behavioural measures were assessed.

Results: LEAP2 concentration was higher in participants with OW/OB ($p < 0.001$) and in females ($p < 0.001$), and was associated with LEAP2 single nucleotide polymorphisms rs765760 ($p = 0.012$) and rs803223 ($p = 0.019$), but not with *LEAP2* methylation status. LEAP2 concentration was directly related to glycaemia ($p = 0.001$) and fullness ($p = 0.003$) in participants with normal weight, whereas it was associated with body mass index ($p = 0.018$), waist circumference ($p = 0.014$) and motor impulsivity in participants with OW/OB ($p = 0.005$). A negative association with reward responsiveness was observed in participants with OW/OB ($p = 0.023$). LEAP2 concentration was inversely associated with food intake ($p = 0.034$) and decreased after a high-protein meal ($p < 0.001$), particularly in women ($p = 0.002$).

Conclusion: Increased LEAP2 in participants with OW/OB is associated with behavioural characteristics of obesity. Our results show sexual dimorphism in LEAP2 concentration before and after food intake and highlight the role of LEAP2 in feeding regulation.

KEYWORDS
feeding behaviour, food intake, impulsivity, LEAP2, reward sensitivity

1 | INTRODUCTION

The liver-expressed antimicrobial peptide 2 (LEAP2) was recognized in 2018 as an endogenous blocker of the growth hormone secretagogue receptor (GHSR),[1,2] the receptor for hormone ghrelin.[3] Circulating LEAP2 appears to behave oppositely to ghrelin, decreasing under energy deficit and increasing after refeeding in mice.[1,4] Notably, LEAP2 displays similar anorectic and insulinotropic effects in humans as seen for glucagon-like peptide 1, a key target in treating type 2 diabetes and obesity (OB).[5,6] Thus, LEAP2 has emerged as a new attractive target to treat metabolic disorders.

Given its recent recognition as a hormone, the dynamics of LEAP2 in human plasma and the underlying molecular mechanisms remain poorly understood. As observed in mice,[1,7] plasma LEAP2 concentration was found to be increased in participants with obesity.[7,8] A positive association of LEAP2 with adiposity and glycaemia has been reported in cohorts comprising participants with normal weight (NW), overweight (OW), or OB.[7,8] Similar findings have been observed in patients with liver steatosis[9] and prediabetes.[10] However, whether these associations are affected by nutritional status remains a matter of debate. Intriguingly, higher circulating LEAP2 concentration has been reported after puberty in girls but not in boys,[11] and genetic deletion of LEAP2 has been shown to protect against OB in adult female but not male mice.[12] However, insufficient attention has been paid to studying sex-specific effects in humans, as most studies were conducted in one sex,[7] lack reports on sex differences,[9,10,13,14] or fail to provide clear evidence.[15] Furthermore, the molecular bases that may influence plasma LEAP2 concentrations remain understudied in humans. We showed that *LEAP2* genetic variants are differentially associated with OB traits[16]; however, the association between plasma LEAP2 concentration and *LEAP2* genetic variants has not been reported. Similarly, while DNA methylation regulates gene expression,[17] the association between LEAP2 plasma concentration and *LEAP2*-related methylation sites has not been studied.

The role of LEAP2 in meal regulation and postprandial LEAP2 concentration changes also remains to be clarified. Few studies have assessed plasma LEAP2 concentration in lean participants following a meal, reporting no changes[7,8,14] or a postprandial increase.[13] However, these studies were performed using standardized, fixed carbohydrate-rich meals, and were focused exclusively on women,[7] participants without OB,[13] or participants undergoing Roux-en-Y gastric bypass.[14] Thus, there is a need for studies evaluating postprandial LEAP2 changes in ad libitum feeding, the usual daily eating condition in modern societies. Postprandial LEAP2 concentration was reported to depend on meal composition in mice, increasing after lipid administration[18]; however, the effect of dietary proteins is still unknown. Recently, we found that preprandial plasma LEAP2 concentration is inversely associated with hunger sensation in a ghrelin-independent manner[8] and another research group reported that the postprandial increase in LEAP2 is associated with a decrease in appetite sensation.[13] However, the associations of LEAP2 with other appetite sensations and eating behaviours such as impulsivity, reward sensitivity, food preferences and hedonic eating remain unexplored. In both animal models and human studies, GHSR has been reported to be involved in different aspects of feeding behaviour,[19,20] while ghrelin concentrations have been associated with hunger,[21] hedonic eating[22–24] and reward sensitivity.[25] Therefore, plasma LEAP2 concentration could be involved in several aspects of feeding behaviour.

Here, we hypothesized that plasma LEAP2 concentration depends on the nutritional status or sex and that it could be associated with various anthropometric, metabolic, and genotypic variables. The present study aims: (a) to investigate the variations in fasting LEAP2 concentrations among participants with different nutritional status (Part 1, Section 2.2.1); (b) to study the relationship between fasting LEAP2 concentration, and anthropometric and metabolic traits, feeding behaviour, *LEAP2* genetic variants and DNA methylation status, and whether sex or nutritional status modulate these associations (Part 1, Section 2.2.1); and (c) to ascertain postprandial changes in LEAP2 concentration after a high-protein intake and the association with feeding behaviour and/or food intake (Part 2, Section 2.2.2). As previous studies have been hindered by limited sample sizes, compromising the robustness and generalizability of findings, we assessed plasma LEAP2 concentration in samples from a large cohort to overcome a limitation of previous research.

2 | METHODS

2.1 | Study participants

Data and plasma samples for LEAP2 assessment originate from a study conducted at Uppsala University.[26] Between August 2011 and August 2018, male and female participants were recruited from public university campuses in Uppsala, Sweden. An interview was performed to ensure participants were in generally good health. Criteria for inclusion were healthy participants aged 18–40 years with normal dietary

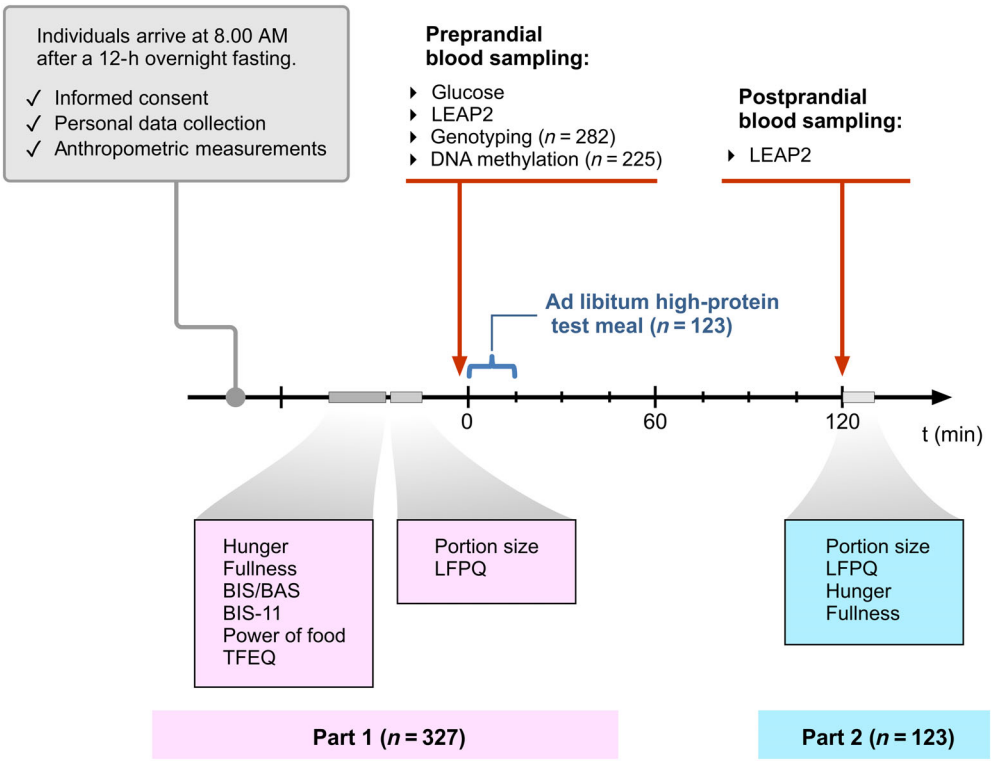

FIGURE 1 Overview of study design. BIS/BAS, behavioural inhibition and activation systems; BIS-11, Barratt impulsivity scale; LEAP2, liver-expressed antimicrobial peptide 2; LFPQ, Leeds Food Preference Questionnaire; TFEQ, three-factor eating questionnaire.

habits and of northern ancestry (grandparents born in Sweden, Denmark, Norway, Finland, or Iceland). Exclusion criteria included participants who were vegetarians/vegans, smokers, lactose or gluten intolerant, or undergoing medical therapy, and pregnant or lactating women (Figure S1).

Ethics approval was granted by the regional ethical review board in Uppsala. Written informed consent was obtained from all participants.

The study was registered on ClinicalTrials.gov (NCT01863212).

2.2 | Study protocol

Figure 1 depicts the study design.

2.2.1 | Part 1

Participants arrived at the Institute in the morning after a 12-h overnight fast and underwent an initial evaluation, including a medical and family history. Education level was assessed by recording years of formal education. Fasted state was confirmed via blood glucose measurements. Weight, height and waist circumference were measured using standard procedures.[27] Body mass index (BMI) was calculated by dividing weight by height squared (kg/m^2). According to World Health Organization criteria, participants were categorized into NW (BMI 18.5–24.99 kg/m^2), and OW or obese (OW/OB; BMI ≥25 kg/m^2).[28] Next, a blood sample was obtained and participants were requested to rate their perceived hunger and fullness on a 100-mm visual analogue scale[29–32] and to complete the Behavioural Inhibition and Activation Systems (BIS/BAS) scale that evaluates sensitivity to reward,[33] the Barratt Impulsivity Scale (BIS-11), and the Power of Food and three-factor eating questionnaires (TFEQ) (see Data S1).

2.2.2 | Part 2

After undergoing a portion size task (measures the portion size ideal for the participant) and completing the Leeds Food Preference Questionnaire (LFPQ) (descriptions in Data S1), some participants underwent an ad libitum test meal in which they were given free access to a high-energy and high-protein breakfast. The breakfast consisted of whole-wheat fruit rolls (Fruktkuse; Coop Sverige AB) with Leerdamer cheese and Quark curd cheese (Arla) (per 100 g: 153 kcal, energy composition: 35.3% protein, 31.3% carbohydrates and 33.4% fat). Participants were told to consume the meal until they felt comfortably full (no longer than 15 min). Water (30 mL) was provided as a beverage. When the participants felt full, the rest of each food item was weighted and energy intake was calculated. A second blood sample was taken 120 min after the first venepuncture. Then, participants were asked to rate their perceived hunger and fullness again, conducted the second portion size task and completed the LFPQ in a postprandial state to assess feeding behaviour. Baseline and postprandial blood samples were collected in tubes containing heparin. Blood samples were also collected in PAXgene tubes at baseline for genotyping and DNA methylation profiling (see Data S1). Samples were stored at −80°C until analysis.

2.3 | Biochemical analyses

Glycaemia was measured using an Accu-Chek Aviva blood glucose meter (Roche). LEAP2 was assessed using an immunoassay from Phoenix Pharmaceutical (EK-075-40, intra- and inter-assay variations of 6.4% and 9.9%, respectively, validated in Mani et al.[7]), following the manufacturer's instructions.

2.4 | Statistics and data analyses

Statistical analyses were performed using the R software version 4.0.3 or SPSS version 28.0. Normal distribution was tested using Shapiro–Wilk or Kolmogorov–Smirnov tests. Variables normally distributed were summarized as mean ± standard deviation (SD), and nonparametric data were presented as median (interquartile range, IQR). Given the cross-sectional design, missing data was kept at a minimum (one value per variable or less) and managed through pairwise deletion. Because of positive skewness, LEAP2 concentration was log-transformed for analyses. In Part 1, differences between groups were analysed using a two-way analysis of variance (ANOVA) with BMI category and sex as independent variables. When the data were non-parametric, a robust two-way ANOVA was used. Multiple linear regression analysis was used to test the relationship of LEAP2 concentration with BMI, waist circumference, glycaemia, behavioural outcomes and LEAP2 genetic variants, adjusting for age, sex, BMI and/or educational level when needed. Interaction analyses were performed to estimate whether sex or BMI modified the association between LEAP2 and the variables assessed. Multiple testing correction was not applied because anthropometric measures and the subscales of behavioural outcomes are highly correlated.[34,35] The distribution of residuals was checked using the Shapiro–Wilk or Kolmogorov–Smirnov tests and normal quantile plots. Regression diagnostics were computed to confirm that assumptions of linear regression were met. Linear mixed-effects models were used to test for pre- and postprandial differences in LEAP2 concentration, adjusting for fixed effects of time (pre- and postprandial), BMI category, age and sex, while treating each participant as a 'unit of analysis' via the random intercept. Analogous models were used to test for pre- and postprandial changes across behavioural measures, adjusting for fixed effects of time, BMI category, age, sex and LEAP2 concentration and treating each participant as a 'unit of analysis' via the random intercept. Power calculations were omitted as these data were derived from a pre-existing clinical trial.

3 | RESULTS

3.1 | Part 1

3.1.1 | Characteristics of participants

The study included 327 participants (128 females and 199 males) with a median age (IQR) of 25[23–27] years and a median BMI (IQR) of 23.88 (21.78–26.29) kg/m^2. Based on BMI, 196 participants were categorized as with NW, and 131 as participants with OW/OB. Table 1 details participant characteristics. Plasma LEAP2 concentration was higher in participants with OW/OB and in females, with no sex × BMI category interaction. No significant differences were observed in hunger, fullness, or the BIS/BAS scale. Females with OW/OB showed higher scores on the BIS-11 motor subscale (sex × BMI category interaction), indicating increased motor impulsivity (failure to think before acting). Hedonic hunger, as per the Power of Food scale, was higher in participants with OW/OB. Females exhibited higher disinhibition and cognitive restraint scores on the TFEQ, with disinhibition also higher in participants with OW/OB.

3.1.2 | Liver-expressed antimicrobial peptide 2 concentration is associated with anthropometric variables, glycaemia and motor impulsivity

Table 2 shows the associations of LEAP2 concentration as a predictor of anthropometric, metabolic and behavioural measures. LEAP2 concentration exhibited a direct relationship with BMI, waist circumference, glycaemia and the BIS-11 motor subscale. However, no associations were found with perceived hunger, fullness, BIS-BAS scale, Power of Food, or TFEQ dimensions. To estimate whether sex or BMI modified the association between LEAP2 and the variables assessed, sex × plasma LEAP2 and BMI × plasma LEAP2 interaction analyses were performed. As no significant interactions between sex and plasma LEAP2 concentration were observed, sex-stratified analyses were not performed. In contrast, significant interactions between BMI and LEAP2 were observed for glycaemia, fullness, the Reward Responsiveness element of the BIS-BAS scale and the BIS-11 motor subscale. When analysing participants with NW and OW/OB separately (Table 2 and Figure 2), LEAP2 concentration displayed a direct relationship with glycaemia and fullness only in participants with NW, and with BMI, waist circumference, and the BIS-11 motor subscale only in participants with OW/OB. A negative association with the Reward Responsiveness element of the BIS-BAS scale was observed only in participants with OW/OB.

3.1.3 | Liver-expressed antimicrobial peptide 2 concentration is associated with genetic variants but not with blood cell liver-expressed antimicrobial peptide 2 methylation status

LEAP2 concentration was positively associated with the presence of the rs803223 G allele [beta (95% CI) rs803223: 0.093 (0.015, 0.171), $p = 0.019$] and the rs765760 T allele [beta (95% CI) rs765760: 0.130 (0.029, 0.230), $p = 0.012$]. We also investigated if LEAP2 plasma levels are related to the methylation strengths of neighbouring CpGs. However, no significant correlations between LEAP2-related CpGs cg02081065, cg05967596, cg11397957, cg12607188, cg26975459,

TABLE 1 Characteristics of participants (study 1)

Characteristics	Female		Male		*p*-Value		
	NW ($n = 88$)	OW/OB ($n = 40$)	NW ($n = 108$)	OW/OB ($n = 91$)	Interaction	Sex	BMIcat
Waist circumference, cm[a]	71.0 (69.0, 74.0)	82.0 (78.2, 87.5)	80.0 (76.0, 83.0)	91.0 (86.0, 98.0)	0.246	**<0.001**	**<0.001**
Glycaemia, mmol/L[a]	4.8 (4.6, 5.2)	4.8 (4.6, 5.1)	5.0 (4.7, 5.4)	5.2 (4.8, 5.4)	0.705	**0.003**	0.593
LEAP2, ng/mL[b]	14.54 (13.20,16.03)	19.73 (17.17, 22.68)	11.98 (11.10, 12.95)	14.12 (12.96, 15.40)	0.166	**<0.001**	**<0.001**
Hunger, cm[a]	6.0 (4.5, 7.0)	6.0 (4.5, 7.0)	6.0 (5.0, 7.0)	6,0 (4.0, 7.0)	0.515	0.825	0.954
Fullness, cm[a]	2.0 (1.0, 3.0)	2.0 (1.0, 4.0)	2.0 (1.0, 4.0)	3.0 (1.0, 4.0)	0.607	0.344	0.873
BIS/BAS							
BAS drive[a]	8.0 (7.0, 10.0)	8.0 (8.0, 9.5)	8.0 (6.0, 10.0)	9.0 (8.0, 11.0)	0.212	0.388	0.609
BAS reward responsiveness[a]	18.0 (16.0, 19.0)	17.0 (16.0, 19.0)	16.5 (15.0, 18.0)	17.0 (15.5, 18.0)	0.853	0.162	0.910
BAS fun seeking[a]	12.0 (10.7, 13.0)	13.0 (11.5, 13.0)	12.0 (11.0, 13.0)	12.0 (10.2, 13.0)	0.357	0.272	0.415
BIS total score[a]	22.0 (19.0, 24.0)	19.0 (15.0, 23.0)	19.0 (17.0, 21.0)	19.0 (15.2, 20.0)	0.323	0.258	0.083
BIS-11							
Attentional[a]	15.0 (13.7, 18.0)	17.0 (13.0, 19.5)	15.0 (13.0, 18.0)	15.0 (13.0, 18.7)	0.696	0.453	0.446
Motor[a]	21.0 (18.0, 23.0)^{A}	23.5 (20.2, 25.0)^{B}	22.0 (20.0, 24.0)^{A}	22.0 (19.0, 24.0)^{A}	**0.031**	0.313	**0.022**
Non-planning[a]	23.0 (21.0, 26.0)	23.0 (19.5, 26.5)	23.0 (20.0, 25.0)	23.0 (21.0, 27.0)	0.558	0.987	0.808
Total[a]	58.5 (54.7, 66.0)	58.0 (55.0, 69.5)	61.0 (55.0, 65.2)	59.5 (56.0, 67.7)	0.424	0.609	0.276
Power of Food[a]	22 (12, 28)	26 (16, 42)	14 (8, 22)	21 (11, 38)	0.385	0.245	**0.018**
TFEQ							
Hunger[a]	6.0 (4.0, 8.7)	7.0 (4.0, 9.0)	4.0 (2.0, 7.0)	6.0 (4.0, 9.0)	0.267	0.163	0.462
Disinhibition[a]	6.0 (4.0, 8.0)	8.0 (5.0, 11.0)	4.0 (3.0, 5.0)	6.0 (4.0, 8.0)	0.288	**0.050**	**0.011**
Cognitive restraint[a]	7.0 (4.0, 12.0)	9.0 (5.0, 13.0)	5.0 (3.0, 8.0)	6.5 (4.0, 10.0)	0.858	**0.050**	0.292

Note: Values in bold represent statistical significance (p<0.05). Data analysis performed by two-way ANOVA with BMIcat and sex as independent variables. *P*-values for sex, BMIcat and sex × BMIcat interaction are shown. Different superscript letters indicate significant differences by Tuckey test after two-way ANOVA.

Abbreviations: BIS/BAS, behavioural inhibition and activation systems scale; BIS-11, Barratt impulsivity scale; BMIcat, body mass index category; NW, normal weight; OW/OB, overweight/obesity; TFEQ, three-factor eating questionnaire.

[a]Data analysis performed by robust two-way ANOVA. Data is presented as median (IQR). *P*-values for sex, BMIcat and sex × BMIcat interaction are shown.

[b]Data analysis performed on natural log-transformed values. Back-transformed values are presented as geometric means and 95% confidence intervals.

cg27440002 and LEAP2 plasma concentration were observed (data not shown).

3.2 | Part 2

3.2.1 | Preprandial liver-expressed antimicrobial peptide 2 concentration is inversely associated with energy intake

Pre-to-postprandial changes in plasma LEAP2 concentration were assessed in some participants ($N = 123$, 49 NW females, 13 OW/OB females, 39 NW males and 22 OW/OB males). Characteristics of this subset (Table S1) were similar to the total participants shown in Table 1. Total energy intake was higher in males compared with females ($p < 0.001$). Adjusting for sex, age and BMI, we found that preprandial LEAP2 concentration displayed an inverse relationship with ad libitum energy intake [beta (95% CI): −41.33 (−79.58, −3.09), $p = 0.034$]. No significant interactions between plasma LEAP2 concentration and sex or BMI on total energy intake were observed ($p = 0.815$ and 0.502, respectively).

3.2.2 | Liver-expressed antimicrobial peptide 2 concentration decreases after a high-protein meal but is not involved in postprandial changes in feeding behaviour

Figure 3A shows changes in LEAP2 concentration after breakfast according to sex and BMI category. Linear mixed-effects models controlling for time (pre-postprandial), sex, age and BMI category showed a significant postprandial decrease in LEAP2 ($p < 0.001$). LEAP2 was higher in females ($p = 0.002$) and in participants with OW/OB ($p = 0.008$), with no age effect ($p = 0.322$). A sex × time interaction

TABLE 2 Associations between LEAP2 concentration and anthropometric, metabolic, and behavioural measures.

	All (1)		Sex × LEAP2 interaction	BMI × LEAP2 interaction	NW (2)		OW/OB (2)	
Plasma LEAP2 concentration, ng/mL	Beta (95% CI)	*p*	*p*	*p*	Beta (95% CI)	*p*	Beta (95% CI)	*p*
BMI, kg/m^2[a]	1.450 (0.715, 2.185)	**0.001**	0.968	-	−0.066 (−0.544, 0.412)	0.786	1.239 (0.217, 2.262)	**0.018**
Waist circumference, cm[a]	4.303 (1.889, 6.717)	**0.001**	0.081	-	−1.411 (−3.187, 0.366)	0.119	4.872 (0.992, 8.752)	**0.014**
Glycemia, mmol/L	0.133 (0.092, 0.120)	**0.035**	0.383	**0.003**	0.267 (0.109, 0.425)	**0.001**	0.019 (−0.182, 0.219)	0.854
Hunger, cm[b]	0.397 (−0.070, 0.864)	0.096	0.142	0.704				
Fullness, cm[b]	0.344 (−0.195, 0.882)	0.210	0.110	**0.018**	0.936 (0.330, 1.541)	**0.003**	−0.717 (−1.772, 0.339)	0.180
BIS/BAS								
BAS drive[b]	−0.214 (−0.998, 0.569)	0.590	0.321	0.228				
BAS reward responsiveness[b]	−0.385 (−1.050, 0.279)	0.254	0.821	**0.021**	−0.100 (−0.766, 0.746)	0.979	−1.319 (−2.451, −0.186)	**0.023**
BAS fun-seeking[b]	0.051 (−0.582, 0.685)	0.873	0.455	0.318				
BIS total score[b]	0.332 (−0.825, 1.488)	0.573	0.545	0.922				
BIS-11								
Attentional[b]	0.537 (−0.457, 1.532)	0.288	0.055	0.781				
Motor[b]	1.137 (0.091, 2.183)	**0.033**	0.907	**0.007**	0.543 (−0.772, 1.858)	0.416	2.809 (0.857, 4.760)	**0.005**
Non-planning[b]	0.302 (−1.105, 1.710)	0.672	0.247	0.840				
Total[b]	1.729 (−1.051, 4.509)	0.958	0.206	0.490				
Power of Food[b]	1.230 (−1.786, 4.246)	0.423	0.187	0.678				
TFEQ								
Hunger[b]	−0.142 (−1.060, 0.799)	0.762	0.349	0.608				
Disinhibition[b]	0.239 (−0.564, 1.043)	0.558	0.659	0.885				
Cognitive restraint[b]	−0.675 (−1.994, −0.643)	0.314	0.414	0.079				

Note: Values in bold represent statistical significance (p<0.05). Gray shades: analysis not performed. Data are presented as the regression coefficient beta (95% confidence interval) from linear regression analyses. 1: adjusted for age, sex and BMI. 2: adjusted for age and sex.

Abbreviations: BIS/BAS, behavioural inhibition and activation systems scale; BIS-11, Barratt impulsivity scale; BMI, body mass index; NW, normal weight; OW/OB, overweight/obesity; LEAP2, liver-expressed antimicrobial peptide 2; TFEQ, three-factor eating questionnaire.

[a]Not adjusted for BMI.

[b]Further adjusted for educational level.

($p = 0.027$) indicated a more pronounced postprandial decrease in LEAP2 concentration in females than in males. Figure 3 also depicts postprandial changes in behavioural measures: hunger (Figure 3B), fullness (Figure 3C) and portion size task (Figure 3D) according to sex and BMI category. Linear mixed-effects models controlling for time, sex, age, BMI category and LEAP2 concentration showed a postprandial reduction in hunger and portion size as well as an increase in fullness (time effect). Portion size and hunger sensation were lower in females (sex effect). No effect of BMI category, age, or LEAP2 concentration was observed. Figure S2 shows the postprandial changes in

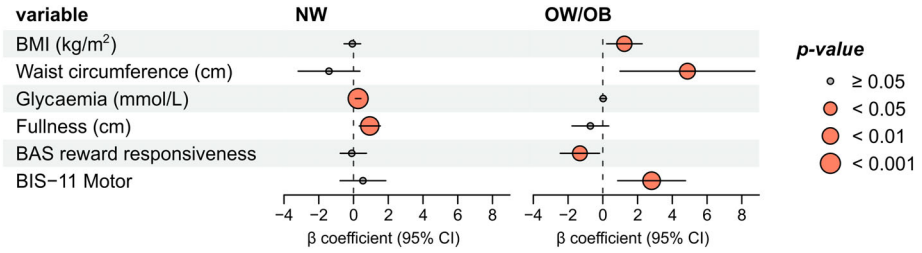

FIGURE 2 Forest plot showing the estimated regression coefficient beta (95% confidence interval) from linear regression analyses for associations between liver-expressed antimicrobial peptide 2 (LEAP2) concentration as a predictor of anthropometric, metabolic and behavioural measures in participants with normal weight (NW) and with overweight or obesity (OW/OB). BAS, behavioural activation systems; BIS-11, Barratt impulsivity scale; BMI, body mass index.

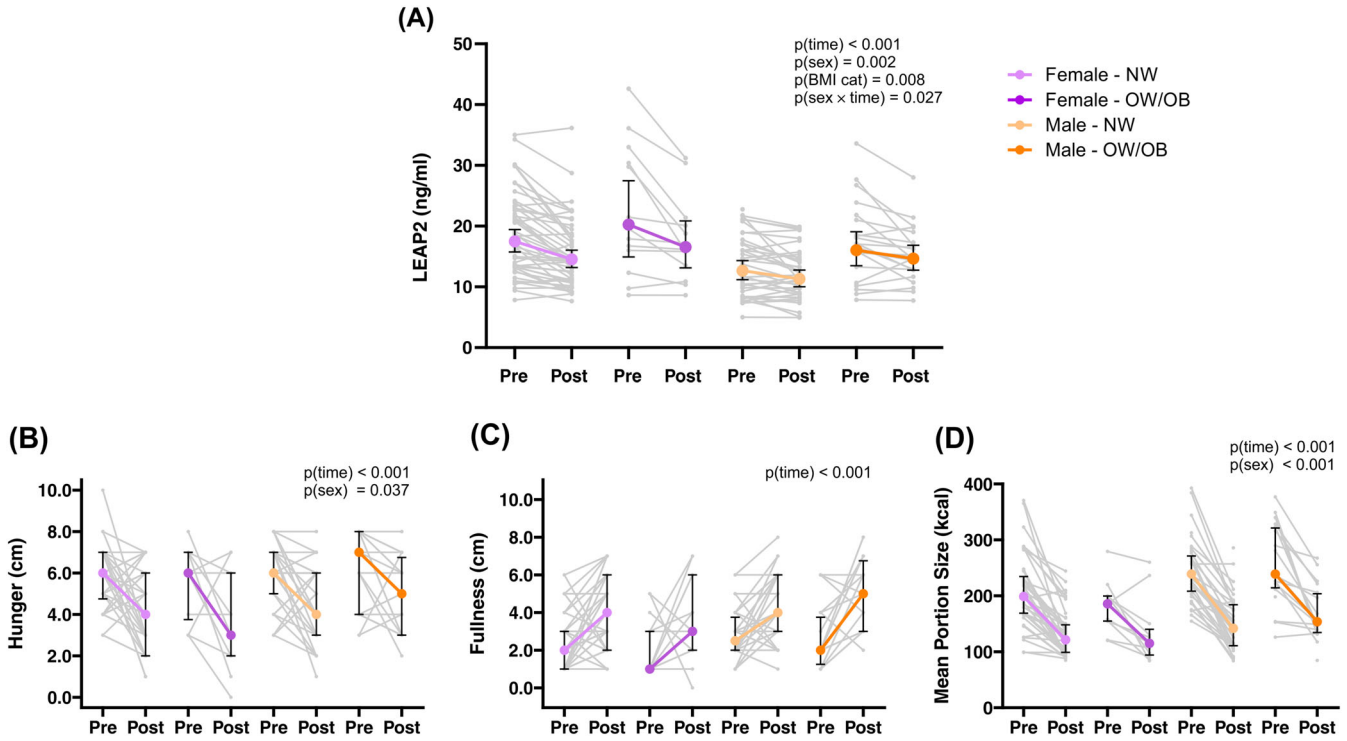

FIGURE 3 Pre- and postprandial (A) liver-expressed antimicrobial peptide 2 (LEAP2) concentration, (B) hunger, (C) fullness and (D) portion size after an ad libitum high-protein test meal according to sex and body mass index (BMI) category. Data were analysed using linear mixed-effects models controlling for (A–D) time (pre- to postprandial), sex, age and BMI category and including (B–D) LEAP2 concentration in the model. Coloured points and lines show the geometric (A) mean and 95% CI or (B–D) median and IQR and each grey line represents the change within each individual participant. Significant *p*-values for the main effects of time, sex, BMI category and for the interaction between sex and time are shown. NW, normal weight; OW/OB, overweight or obesity.

explicit liking (Figure S2A) and wanting (Figure S2B) scores for HFSA, LFSA, HFSW and LFSW foods in all participants. Linear mixed-effects models showed a postprandial reduction in all measures except explicit liking and wanting of LFSW foods. Explicit liking of HFSA, HFSW and LFSW foods was lower in females. No effects of BMI category, age, or LEAP2 concentration were observed.

4 | DISCUSSION

To our knowledge, this study is the first to assess variations in fasting LEAP2 concentrations and their underlying regulatory mechanisms within a cohort with comprehensive data. Our findings revealed a putative role of LEAP2 in fullness, motor impulsivity and reward responsiveness inherent in OB, while also unveiling a robust association between LEAP2 concentration and *LEAP2* genetic variants, but not to its methylation status. Our research underscores that preprandial LEAP2 levels are inversely associated with energy intake, and that the consumption of ad libitum high-protein meals, particularly among females, leads to a decrease in LEAP2 concentration.

To our knowledge, this study is the first to report outcomes in a large population of men and women, as fasting LEAP2 concentrations have been investigated in diverse small cohorts.[7–9] Our results show that LEAP2 concentration is higher in females and is directly

associated with BMI, waist circumference and glycaemia, consistent with previous reports.[7–9] Importantly, the large cohort of participants allowed us to reveal that the association between LEAP2 and some variables is modulated by the nutritional status. Specifically, the association between LEAP2 concentration and BMI and waist circumference is present only in participants with OW/OB, independently of sex. Further investigations are needed to establish whether an elevated LEAP2 concentration has a primary role in the onset of OB or whether it increases as a consequence of fat accretion. Conversely, a direct association of LEAP2 with glycaemia was observed only in participants with NW, suggesting that LEAP2 may contribute to glucose homeostasis in individuals without OB.

Our data provide insight into the molecular basis affecting LEAP2 concentration. In particular, we found that LEAP2 common genetic variants rs803223 (MAF 25.6%) and rs765760 (MAF 20.2%) are associated with LEAP2 concentration, independently of sex and BMI, indicating that these genetic variants may influence *LEAP2* gene expression. To our knowledge, this is the first work linking LEAP2 genetic variants with LEAP2 concentration. In addition, we recently reported that the rs803221 variant of the LEAP2 gene, which is dependent to rs803223 according to linkage disequilibrium, is associated with OB-related traits.[16] Thus, these associations may be concomitant with changes in the peptide plasma concentration. Conversely, we found no association between plasma LEAP2 levels and its related CpGs. While the methylation status of blood cells can reflect the epigenetic signatures of biologically relevant target tissues,[36] our findings indicate that it does not influence LEAP2 gene expression.

We found here that LEAP2 concentration is inversely associated with reward sensitivity only in participants with OW/OB, indicating that higher concentrations of LEAP2 may decrease responsiveness to rewarding stimuli such as palatable foods, a characteristic of OB.[37] Differences in sensitivity to reward have been long recognized,[38] and dysregulation of the reward system (greater sensitivity to reward) has been linked to different psychological disorders, including eating disorders.[39,40] Consistent with our observation, LEAP2 administration in mice attenuates binge eating for high-fat diet,[19] the preference for peanut butter,[41] and alcohol intake,[42] giving further support to the role of the GHSR system in the incentive value of some stimuli.[20] In contrast, LEAP2 concentration positively associates with motor impulsivity, which is higher in women with OW/OB and is linked to overeating and OB.[43] This relationship was observed in the entire cohort and specifically in participants with OW/OB, indicating that LEAP2 may modulate the failure to think before acting, a characteristic of participants with OB. Importantly, LEAP2 was associated with general reward and impulsivity measures (BIS/BAS and BIS-11 scales) but not with eating-specific measures such as hedonic hunger and eating behaviour assessed using the Power of Food scale or the TFEQ. Therefore, LEAP2 could be related to personality traits influencing eating, reward mechanisms or impulsivity.

Our observation that the preprandial LEAP2 concentration inversely associates with ad libitum energy intake, highlights the role of this peptide in feeding regulation in humans. Consistent with the current observations, our previous results[8] show an inverse association of preprandial LEAP2 concentration with hunger. Similar results were observed in participants offered a high-energy meal,[13] and a reduction of food intake was observed after LEAP2 administration that achieves mildly supraphysiological plasma LEAP2 concentrations.[6] However, previous studies were performed only in lean men.[6,13] Our results show that the negative association of LEAP2 and energy intake is independent of sex and BMI, and support a role for GHSR signalling in food intake regulation, either through ghrelin antagonism or ghrelin-independent actions on GHSR.[44] As current evidence indicates that LEAP2 exclusively acts by downregulating GHSR signalling,[6] it seems probable that LEAP2 affects eating, metabolism and reward signalling by regulating the same neuronal circuits as ghrelin. However, further studies are required to clarify how the opposite actions of this dual hormonal system are integrated at the central level.

Our findings indicate that some implications of LEAP2 depend on the nutritional status. The associations of LEAP2 concentration with anthropometric variables, reward sensitivity and impulsivity were detected only in participants with OW/OB, suggesting that increased LEAP2 in participants with OW/OB plays an important role in fat accretion and modulates some of the behavioural characteristics of OB. LEAP2's inverse association with ad libitum energy intake, independently of sex and BMI, contrasts with its association with fullness sensation and glycaemia observed only in lean participants, suggesting that the role of LEAP2 in satiety and glucose metabolism may be altered in participants with OW/OB. Reduced sensitivity to appetite-regulating hormones has been widely reported, mainly in animal models of OB, but requires further investigation for LEAP2.

To our knowledge, this study is the first evaluation of LEAP2 concentration after an ad libitum high-protein meal (35.3%). Previous studies, from our group and others, provided test meals containing between 12% and 18% of energy as proteins,[7,8,13,14] finding increased or unchanged postprandial LEAP2 concentrations, as was also observed for oral glucose and lactate administration.[45] A mouse study examining LEAP2 after different acute meal challenges found that hepatic *Leap2* expression correlates with hepatic glycogen levels, indicating that high carbohydrate-induced accumulation of liver glycogen is needed to increase *Leap2* expression.[18] Here, we found a postprandial decrease in LEAP2 concentration. Consistent with our results, a recent study assessing the mouse hepatic transcriptome found that *Leap2* expression decreased with high-protein meals (>20%).[46] The impact of postprandial LEAP2 reduction on energy balance and subsequent meal intake remains to be elucidated. Analysis using linear mixed-effects models revealed that the postprandial LEAP2 concentration decrease was more pronounced in women than in men, in line with previous results from our group showing a significant postprandial change after a fixed carbohydrate-rich meal only in women with OW/OB.[8] Our findings indicate sexual dimorphism regarding not only preprandial LEAP2 concentration but also in response to food intake. It could be hypothesized that oestrogen, being a key regulator of energy

balance, can modulate LEAP2. In addition, sex differences in insulin response after meals could affect LEAP2 response.

The postprandial reduction in LEAP2 concentration after an ad libitum high-protein meal was not associated with changes in hunger sensation or hedonic feeding represented by the LFPQ and the portion size task. Although the preprandial LEAP2 concentration was associated with feeding behaviour, including fullness, this relationship was not evident after food intake. Our results suggest that LEAP2 concentration is associated with feeding behaviour in an energy-deficit status such as fasting, but not after feeding.

The strengths of the present study include the large sample size and the implementation of genotyping and methylation profiling, which allowed us to assess the molecular basis affecting LEAP2 concentration in pre- and postprandial conditions. The limitations include the lack of body composition measurements, relying on waist circumference as a surrogate of adiposity, and the inability to measure plasma insulin and ghrelin concentrations. Further, visual analogue scale scores were assessed using a discrete scale, which is less accurate than a continuous scale,[47] and may lead to a loss of sensitivity, particularly for the measurement of hunger. In addition, our study did not specifically recruit participants with OB; therefore, the OW/OB group was composed mainly of participants with a BMI of <33 kg/m^2. The unbalanced number of participants in each group, particularly in the test meal, is also a limitation of our study. Finally, we could only take one postprandial blood sample.

In summary, our study unveils intricate associations between LEAP2 concentrations, genetic variants and anthropometric measures, modulated by nutritional status. In addition, it clarifies its role in feeding behaviour across fasting and postprandial states. These findings underscore the multifaceted nature of LEAP2 in OB and feeding behaviour regulation, setting the stage for further exploration into its mechanistic underpinnings and therapeutic implications through the use of this peptide, or synthetic mimetics, to downregulate both ghrelin-dependent and independent actions of GHSR and achieve some health benefits.

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, ALK, GR, AS, PNDF and MP. All authors approved the submitted and published versions.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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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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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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