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RESEARCH ARTICLE

Ahmed et al: Hormones, obesity, and lean phenotype

Hormonal predictors of the lean phenotype in humans

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ABSTRACT

Clinical obesity is characterized by excessive fat accumulation and an increased risk of numerous associated comorbidities. Adipose tissue secretes leptin and other adipokines, which play key roles in regulating energy balance, glucose homeostasis, and body fat mass. Recently, incretin and pancreatic hormones have also been shown to influence these processes. However, the regulatory mechanisms and interactions among these hormones are not yet fully understood. This study investigates hormonal predictors of the lean phenotype (in terms of total body fat) in patients undergoing body contouring surgery, with or without prior bariatric surgery. This prospective quasi-experimental study included patients who underwent body contouring procedures at Hamad General Hospital between January 2021 and December 2023. Patients were assessed at three time points: before surgery, 2–3 weeks post-surgery, and 6–10 weeks post-surgery. Body composition and hormone levels were measured, and statistical analyses—including descriptive statistics and logistic regression models—were used to examine trends and predict the lean phenotype. Among the hormones analyzed, amylin showed a significant association with the lean phenotype while increasing leptin GIP and spexin levels negatively modulated the amylin effect. History of bariatric surgery weakly predicted the lean phenotype after adjusting for leptin and gut hormone levels. A margins plot demonstrated the interactions between amylin, spexin, GIP, and leptin levels that collectively predicted the probability of exhibiting the lean phenotype. These findings highlight amylin, GIP, leptin, and spexin as key hormonal predictors of fat mass, underscoring the critical role of gut hormones and adipokines in determining body fat distribution and the lean phenotype in humans.

Keywords: Clinical obesity; adipokines; gut hormones; pancreatic hormones; body contouring surgery; fat mass regulation; amylin; gastric inhibitory polypeptide; GIP; leptin; spexin.

INTRODUCTION

Clinical obesity is a chronic disease characterized by the buildup of excessive fat deposits, posing serious effects on health, and increasing the risk of developing multiple comorbidities [1]. Adipose tissue, in addition to being an efficient energy resource, is considered an endocrine organ that secretes various hormones known as adipokines, which play a major role in maintaining energy balance and glucose homeostasis. Excess adipose tissue has been associated with insulin resistance (IR), defined as a decreased response of insulin receptors to normal insulin levels leading to insulin hypersecretion, which has been linked to several comorbidities including metabolic syndrome, predisposing patients to cardiovascular and metabolic dysfunction–associated steatotic liver disease [2] . Despite the known impact of the adipokines on metabolism and glucose homeostasis, the regulation, and interactions of these hormones with other signaling molecules such as incretin and pancreatic hormones are complex and not fully understood [3]. Recently, researchers have gained interest in incretin and pancreatic hormones due to their impact on glucose homeostasis and weight regulation, complementing the established roles of pancreatic hormones. Understanding the interactions among these adipokines, pancreatic, and incretin hormones remains of high importance and warrants further investigation [3].

Adipokines represent a group of hormones secreted by adipocytes that play a major role in metabolism. Of particular importance is leptin, a critical adipokine that plays a key role in fat mass regulation [4–6]. It is mainly secreted from adipocytes and its plasma concentration increases in proportion to body fat mass. Circulating leptin penetrates the blood-brain barrier to exert its functions on the central nervous system, specifically the mediobasal part of the hypothalamus [7].

In addition to adipokines, gut-derived hormones such as incretins are key regulators of metabolism and energy balance. The primary incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are secreted from the intestinal enteroendocrine cells and enhance glucose-dependent insulin secretion from pancreatic β-cells [8]. Beyond their well-established role in glycemic regulation, GLP-1 receptor agonists (GLP-1 RAs) exert direct effects on adipose tissue, contributing to reductions in fat mass through multiple mechanisms. GLP-1 signaling influences adipose metabolism by modulating lipolysis,

adipogenesis, and thermogenesis. Furthermore, GLP-1 signaling in the hypothalamus suppresses orexigenic pathways while activating anorexigenic circuits, leading to reduced caloric intake, which indirectly facilitates fat loss. Tirzepatide, a dual GLP-1/GIP receptor agonist, has demonstrated superior efficacy in promoting weight loss and improving glycemic control in patients with type 2 diabetes and obesity, potentially due to additive or synergistic effects on lipid metabolism and adipose tissue remodeling [9,10].

Given that incretins exert their effects primarily on pancreatic β-cells, it is important to consider other hormones secreted by these cells that contribute to metabolic regulation. Insulin, the key hormone for glucose homeostasis, is co-secreted with amylin [11]. Amylin plays a major role in inhibiting glucagon secretion from α cells slowing gastric emptying and promoting satiety. The interactions and mechanisms linking these hormones remain of great interest and are not fully understood [12].

Patients undergoing body contouring surgery with or without a history of bariatric surgery present a unique model to explore the interplay between body fat mass and hormone expression. Examining hormone levels in the same patients before and after removal of large volumes of fat enables us to investigate how changes in fat mass influence the regulation of adipokines and gut hormones. These findings may offer valuable insight into how these hormones predict and maintain a lean phenotype in humans. Thus, we aim to assess the interplay between these hormone levels and total body fat% in patients pre and post body contouring surgery who may or may not have had prior bariatric surgery.

MATERIALS AND METHODS

Study population

We investigated patients who underwent body contouring surgeries at the Department of Plastic Surgery at Hamad General Hospital during a period from January 2021 to December 2023. In this prospective quasi-experimental study design, subjects were prospectively followed up at three-time points; within 1 week before the surgery, within 2-3 weeks after the surgery, and within 6-10 weeks after surgery to assess variables of interest. These time points were selected to capture immediate as well as delayed changes in hormonal profile after surgery. The inclusion criteria include patients undergoing surgical subcutaneous fat removal (SSFR) (as known as body contouring surgery), including abdominoplasty and lower body lift or thigh lift

(thighplasty) surgeries with a body mass index (BMI) ≥18 and age ≥18 years old. The exclusion criteria included patients who underwent bariatric surgery less than 18 months before the body contouring surgery, patients with co-morbidities (except diabetes not on pharmacotherapy) or with diabetic nephropathy, patients with body contouring surgeries in areas other than abdomen or thigh and patient older than 65 years or has a BMI over 35. Informed consents were obtained from all study participants prior to inclusion in the study protocol.

Ethical approval

The study received institutional review board approval (IRB) from Medical Research Center at Hamad Medical Corporation under reference MRC-01-20-466.

Assessment of body composition

Body composition was assessed at the defined time points (before and after the surgery) using Tanita body composition analyzer (DC-360 P) that uses bioelectrical impedance analysis (BIA) technology to provide a detailed full body composition analysis [13]. The measured variables are weight, body fat percentage, body fat mass, BMI, fat-free mass, estimated muscle mass, total body water, visceral fat rating (VFR), and basal metabolic rate (BMR). Tanita works by sending low and safe electrical signals from the four metal electrodes; the signal passes faster in water (hydrated muscles) and meets resistance when it passes through fat tissue. Signals outcome is calculated using scientifically validated Tanita equations to create the final report [14].

Lean phenotype

In this study, body fat percentages measured through Tanita were divided into tertiles. Subjects within the lowest tertile were defined as the lean phenotype group, highlighting the distinction in body fat mass.

Oral glucose tolerance test (OGTT)

Fasting Glucose levels were measured in the morning after fasting for at least 8 hours; then 75 grams glucose test was performed with serum glucose measurements at 15, 30, 45, 60 and 120 minutes using a fast multi-assay analyser (Analox-GL5).

Glycemic indices

Homeostatic model assessment was made for each subject at the defined 2 time points (before and after the surgery) using the University of Oxford HOMA2 calculator which estimates steady state beta cell function (%B) and insulin resistance (IR), as percentages of a normal reference population [15].

Body contouring surgeries

Subjects who fulfilled the inclusion criteria underwent abdominoplasty, lower body lift and/or thigh lift surgeries performed as standard. All procedures were performed by expert surgeons in the department.

Hormonal measurements

Plasma and serum samples were collected at the recruitment center by the recruitment staff, aliquoted, and stored at -70°C until further analysis. Samples were transferred at the time of analysis to an adjacent laboratory within the center for hormonal assay. The multiplex panel contained nine hormones (Gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), Pancreatic Peptide (PP), PYY, Amylin, Leptin, Insulin, C-Peptide, Secretin) and only those that were selected into the analysis of this study are reported. These hormones were assessed using EMD Millipore's MILLIPLEX® Human Metabolic Hormone Panel V3. Using the Luminex xMAP technology, this kit enables the simultaneous analysis of the aforementioned analytes in human serum, plasma and tissue/lysate and culture supernatant samples. In addition, spexin and Liver Expressed Antimicrobial Peptide 2 (LEAP2) were assessed using ELISA Kits manufactured by Abbexa Ltd. Samples were assayed in duplicates in one single assay to exclude inter-assay variations. Intra-assay variations were less than 10%. The MILLIPLEX® Human Metabolic Hormone Panel V3 assay exhibits no or negligible cross-reactivity between the antibodies for each analyte and other analytes in the panel, ensuring high specificity for the target hormones. The spexin ELISA Kit is optimized for detecting native spexin, offering a sensitivity of 46.9 pg/mL and a detection range of 78.13 pg/mL to 5000 pg/mL. Similarly, LEAP2 ELISA Kit is designed for detecting native LEAP2, with a sensitivity of < 0.07 ng/mL and a detection range of 0.156 ng/mL to 10 ng/mL.

Sample size

Sample size calculations were not done because they require knowledge of the true effect in the study, which is always unknown (not only before but also after the study is conducted), and which, if known, would make conducting the study unnecessary [16]. Further, post hoc assessments of power were not done because they are deeply problematic (e.g., they are irrelevant and typically are biased and have large sampling variation) and thus were not calculated [17–20]. Instead, in this paper, we included all participants who were available within the time frame of the study.

Statistical analysis

Descriptive statistics regarding patient demographics and variables of interest were reported by time points (preoperative and postoperative). Difference between the time points were calculated for each variable. Trends in hormonal parameters of interest were analyzed in regression models using visits (1-3), bariatric status (RYGB/SG/None) and demographic characteristics as covariates. Logistic regression models were used to predict the lower tertile of body fat mass % which will henceforth be labeled the *lean phenotype*. The correlational structure of repeated measurements in the same patient over time was addressed using cluster robust standard errors. To better understand the relationships, a margins plot was created to depict the results indicated by logistic regression. To determine if the study data are consistent with a population model that assumes no effect, a *p* value was computed [21]. The exact *p* value is reported and tells us the degree of significance (divergence) of the estimated effect from the null hypothesis, had it been the source of the study data. Results in the interval $p<0.05$ were labeled 'statistically significant (divergent)' [21]. To assess clinical benefits, the point estimate and its 95% uncertainty interval (95% UI, formerly known as 95% confidence interval) for potentially data generating null hypotheses were reported with an assessment of the practical importance of the study result. All analyses were conducted using Stata Version 17 (StataCorp, CollegeStation, TX, USA).

RESULTS

Participants' characteristics

Subjects attended visits 1, 2 and 3 were 34, 22 and 27, respectively. BMI, fat% (Tanita), history of bariatric surgery, and hormone levels (leptin, spexin, GLP-1, GIP, PP, Amylin, LEAP2) were compared across the three visits (Table 1). The gender distribution remained consistent across visits, with females comprising the majority at all visits. BMI values showed slight fluctuations across visits, with no significant differences observed. Fat percentage demonstrated reduction in visits 2 and 3 compared to the baseline visit. However, no statistical significance was detected. In addition, the patients' history of bariatric surgery did not show statistically significant alterations across the 3 visits. Median levels of leptin and spexin exhibited fluctuations across visits. Spexin values demonstrated a consistent increasing trend, while leptin levels showed a slight reduction at visit 2 followed by an increase at visit 3. However, these changes were not statistically significant. In contrast, GLP-1, GIP, PP, LEAP2 and Amylin levels demonstrated

statistically significant variations across visits with an initial increase in visit 2 followed by reduction in visit 3 indicating noteworthy changes over time.

Predictors of lean phenotype

When predictors of lean phenotype were analyzed using logistic regression (Table 2), there was a clinically significant *negative* association of lean phenotype with serum leptin (p= 0.026), GIP (p= 0.052) and spexin (p= 0.105) levels. Although some of the p values do not reach the threshold for statistical significance, they are low enough to imply a statistical trend which remains clinically relevant. For each unit increase in leptin, the odds of lean phenotype *decreased* by approximately 10%. On the other hand, there was an increase in lean phenotype with increase in serum amylin and history of bariatric surgery after accounting for the impact of leptin, and gut hormones (GIP and amylin). A margins plot demonstrated that the probability of lean phenotype was mainly predicted by amylin and the other hormones served to increase the threshold for the amylin effect on fat mass% (Figure 1).

The only predictor of leptin sensitivity (a proxy for lower tertile leptin levels) was fat mass % with greater fat mass being associated with increase in serum leptin that we took to indicate decrease in leptin sensitivity [22]. In addition, immediately after SSFR there was no change in leptin sensitivity with sudden loss of fat mass but at visit 3 there was a decline in leptin sensitivity (Figure 2).

DISCUSSION

This study examined the influence of the levels of gut hormones (amylin and GIP) and adipokines (spexin and leptin) on prediction of the lean phenotype in a diverse cohort of patients undergoing body contouring surgery. While individual hormones such as leptin, amylin, GIP and GLP-1 are known to regulate metabolism, the combined influence of these hormones in predicting body fat mass, particularly a lean phenotype, remains poorly understood. Most previous studies have primarily focused on individual hormones in isolation, leaving the combined influence of these hormones unexplored. This study addresses this gap by investigating how total body fat mass influences these hormones and their potential role in predicting a lean phenotype. Body contouring surgery provides a unique model for studying this relationship through pre- and post-surgical comparisons. By examining hormone levels before

and after surgical excision of the subcutaneous fat, we aimed to identify dynamic patterns of hormones levels associated with body fat mass reduction.

The results of this study highlight, for the first time, that amylin, GIP, leptin and spexin predict the lean phenotype, in these participants, robustly. Rising levels of amylin and declining levels of all other hormones predicted the lean phenotype. These results suggest that a combination of gut hormones (amylin and GIP) and adipokines (spexin and leptin) is associated with the lean phenotype in humans. While we demonstrate associations, we cannot be certain that prediction of body fat through hormonal levels in serum is indicative of cause and effect. However, a look at the literature provides compelling evidence that these hormones play a critical role in the body fat regulation and phenotype.

First, it has been reported that amylin is associated with lipolysis in humans. Amylin is a hormone produced in pancreatic β-cells, is well known for its role in regulating food intake and body weight [23–25]. In animal studies administration of amylin has shown a significant reduction in food intake in a dose-dependent manner, with reductions of over 30% lasting up to 24 hours without compensatory hyperphagia [26–28]. This was also demonstrated when an amylin receptor antagonist was injected into rats, resulting in an increase in food intake [29,30]. Furthermore, amylin administration has been shown to reduce body weight and decrease fat deposition [31–33]. Conversely, opposite findings were observed when an amylin receptor antagonist was injected into rats [34].

Second, several clinical studies have reported the effects of the amylin analogue pramlintide on weight control. In diabetic patients (both type 1 and type 2) undergoing insulin therapy, pramlintide treatment resulted in a significant reduction in body weight [35–39]. Similar results were seen a randomized controlled trial conducted on obese subjects not on insulin therapy, where pramlintide treatment for 16 weeks without concomitant lifestyle intervention led to a significant weight reduction ($3.7 \pm 0.5\%$, $P < 0.001$; 3.6 ± 0.6 kg, $P < 0.001$) and a decrease in waist circumference [40]. A long-acting amylin analogue (AM833) has shown promise for obesity treatment, with a dose-dependent progressive decrease in body weight of 6% to 10.8%, over 26 weeks [41]. Given the strong evidence that amylin plays a critical role in body weight regulation, it is plausible that the increased levels of amylin observed in the lean phenotype group contributes to the observed phenotype through mechanism such as reduced food intake,

delayed gastric emptying, and enhanced satiety [28]. However, sustained long-term fat reduction with amylin is less compared to other therapies such as GLP-1 receptor agonists. Our results suggest that amylin's effect may depend on favorable levels of GIP, spexin, and leptin, indicating that the interaction among these hormones could be critical for achieving optimal body fat reduction.

Third, it has been reported that GIP had a strong negative relationship with the threshold at which the amylin effect was seen. This aligns with data suggesting that GIP antagonism induces weight loss. Preclinical studies have shown that the inhibiting GIP receptors improves insulin sensitivity and reduces obesity [42,43]. Wild-type mice fed a high-fat diet developed GIP hypersecretion, extreme fat deposition and insulin resistance, while mice lacking GIP receptors were protected from these effects [42]. Another study investigated the effect of GIP receptors chemical ablation on aspects of obesity-related diabetes [43]. Receptor ablation led to significant reduction in glucose and insulin levels in response to feeding and improved insulin sensitivity. In addition, it helped in correcting obesity-related islet hypertrophy and β-cell hyperplasia. Interestingly, chronic administration of GIP receptor agonist has shown similar effects to GIP receptor antagonist due to receptor desensitization on adipocytes [44,45]. This desensitization likely occurs through the receptor internalization and degradation pathways, reducing GIP signaling over time. Recent studies indicate that human GIP receptor desensitization involves internalization and down regulation, whereas rodent models may follow alternative pathways including second messenger-dependent kinases [46]. Current clinical data support this hypothesis as Tirzepatide, a dual GLP-1 and GIP receptor agonist, has shown superior efficacy in both glycemic control and weight loss compared to other approved medication [9,47]. This effect is thought to result from the synergistic actions of GLP-1 and GIP, with chronic GIP agonist exposure promoting receptor downregulation, mimicking aspects of GIP antagonism while enhancing insulin sensitivity and fat metabolism [9,46].

Fourth, leptin also had a similar effect to GIP in terms of increasing the threshold at which amylin began to predict the lean phenotype. Leptin, a hormone secreted from the adipose tissue, plays a vital role in maintaining energy homeostasis. Higher leptin levels are associated with higher leptin resistance, a marker of the obese state [48,49]. Preclinical and clinical studies have shown that leptin and amylin exert synergistic effects on weight loss. In rats, concurrent

administration of leptin and amylin had an additive effect on food intake suppression and weight loss [50,51]. In human studies, co-administration of recombinant human leptin and the amylin analog (pramlintide) resulted in a mean weight loss 12.7 ± 0.9% (11.5 ± 0.9 kg) significantly higher than either treatment alone [50]. This suggests that restoring leptin sensitivity enhances the fat-reducing effect of amylin. Notably, studies show that amylin improves leptin signaling in the hypothalamus particularly within the ventromedial hypothalamus, reducing hypothalamic inflammation and promoting sustained weight loss [52].

Our findings therefore suggest that the fat-reducing effect of amylin is negatively modulated by increases in leptin, GIP and spexin, thus impairing the effect of amylin. In a state of extreme obesity, leptin resistance, characterized by impaired hypothalamic signaling, neuroinflammation, and receptor desensitization may reduce the ability of exogenous leptin to enhance fat metabolism [53]. This impaired signaling may explain why there could be diminished synergy with amylin in obesity [54,55]. In severely obese rats, amylin effectively reduced body weight and fat mass, but the addition of leptin provided no additional benefit, suggesting that leptin resistance cannot be overcome by exogenous leptin. Notably, caloric restriction induced weight loss did not restore the leptin effect and may only serve to enhance the amylin effect [55], with the diminished response perhaps reflecting chronic leptin receptor desensitization or altered hypothalamic signaling pathways after a state of extreme obesity. However, contradictory results have been reported, suggesting that the interaction between these hormones is influenced by factors such as body weight status, leptin sensitivity, and specific dose regimes [54]. These findings highlight the complexity of the amylin-leptin relationship and underscore the need for further investigation to clarify the underlying mechanisms [50].

This study provides a novel exploration of the interplay among gut and pancreatic hormones, and adipokines, and their prediction of the lean phenotype in the context of body contouring surgery induced changes in fat mass. The study's strength lies in its diverse cohort with varying body fat mass, which enhances the generalizability of the findings. The use of robust statistical models, including logistic regression and cluster robust standard errors further strengthens the validity of the results by accounting for repeated measures and potential covariates. Cluster robust standard error helps minimize bias related to correlated data within the same patient across multiple time points, ensuring more reliable estimates. Despite these strengths, the observational nature of the

study limits its ability to establish causality. This raises the possibility of reverse causation or residual confounding. Furthermore, the relatively small sample size, particularly in the post-surgery group, may reduce the statistical power to detect subtle differences, though the associations found were strong despite the sample size. The variability in hormonal levels and the potential confounding factors associated with surgical procedures, such as surgical stress, postoperative recovery, and medication use, add additional complexity to the interpretation of results. Future studies with larger sample sizes and longitudinal designs are necessary to validate these findings and clarify the directionality of the observed association. Incorporating additional time points and capturing long-term post-surgical hormonal dynamics may provide more comprehensive insights into the complex relationship between these hormones and body fat regulation.

CONCLUSION

This study demonstrates a strong association between serum amylin and the lean phenotype. The modulation of amylin's fat-regulatory effects may be influenced negatively by increases in serum levels of leptin, GIP and spexin, and together, these interactions strongly predict the lean phenotype (figure 3). The lack of predictive value for GLP-1 in this model suggests that GLP-1-related weight loss may involve indirect mechanisms through modulation of hormones such as amylin or leptin. These findings underscore the importance of hormonal interactions in understanding and managing obesity and related metabolic conditions. Future research should focus on confirming these associations in larger longitudinal cohorts while elucidating the underlying mechanisms to better establish causality which can then inform more targeted therapeutic interventions.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Baseline characteristics of participants at each visit.

Factor	Baseline at visit 1 [median (IQR)]	Visit 2 [median (IQR)]	Visit 3 [median (IQR)]	p-value

N	34	22	27	
Gender				
F	27 (79.4%)	16 (72.7%)	22 (81.5%)	0.75
M	7 (20.6%)	6 (27.3%)	5 (18.5%)	
Age	41.5 (37, 47)	35.5 (34, 37)	43 (37, 49)	0.44
BMI	31.6 (26, 33.2)	30.2 (28.4, 33.3)	30.8 (27.2, 33)	0.85
Fat %	37.1 (32.2, 41.9)	34.1 (32, 36)	34.9 (30.9, 39.3)	0.14
HOMA-S%	73.4 (61.2, 82)	67.3 (53.9, 85.3)	66.0 (57.7, 84.9)	0.89
History of Bariatric surgery				
None	16 (47.1%)	11 (50.0%)	17 (63.0%)	0.38
Gastric Bypass	6 (17.6%)	1 (4.5%)	2 (7.4%)	
Sleeve Gastrectomy	12 (35.3%)	10 (45.5%)	8 (29.6%)	
Leptin (µg/L)	9.4 (6.2, 18.6)	7.7 (5, 14.9)	11.2 (8.1, 18.1)	0.32
Spexin (pg/ml)	244.3 (144.6, 457.8)	266.1 (134.3, 469.1)	280.9 (141.8, 374.3)	0.95
GLP-1 (pg/ml)	169.8 (99.2, 329.4)	**298.7 (179.2, 539.9)**	123.3 (103, 180.5)	**<0.001**
GIP (pg/ml)	89 (32.7, 186.5)	**194.8 (136.7, 229.1)**	37.2 (27.1, 56.1)	**<0.001**
PP (pg/ml)	88 (50, 144.3)	**140.3 (56.7, 253.5)**	62 (36.6, 99.7)	**0.02**
LEAP2 (ng/ml)	4.4 (0.9, 11.7)	**11.6 (10.4, 14.2)**	0.8 (0.7, 1.2)	**<0.001**
Amylin (pg/ml)	10.1 (0.0, 22.4)	**17.8 (9.2, 36)**	10.1 (6.6, 13.1)	**0.03**

Sample size (N), Female (F), Male (M), Inter quartile range (IQR), Gastric inhibitory polypeptide (GIP), glucagon-like peptide-1 (GLP-1), Pancreatic Peptide (PP), Liver Expressed Antimicrobial Peptide 2 (LEAP2).

Table 2. Predictors of the lean phenotype using logistic regression.

Lower tertile fat%	OR	P>|z|	95% uncertainty interval
Visit			
1	1 (base)		
2	1.377	0.733	0.218, 8.681
3	1.540	0.522	0.411, 5.775
History of bariatric surgery			
No	1 (base)		
BP	3.660	0.308	0.302, 44.361
SL	1.250	0.754	0.311, 5.025
Amylin pg/mL	1.098	**0.031**	1.009, 1.196
GIP pg/mL	0.989	0.052	0.978, 1.000
Leptin ug/L	0.894	**0.026**	0.811, 0.987
Spexin pg/mL	0.997	0.105	0.994, 1.000
Constant (baseline odds)	1.110	0.914	0.167, 7.384

*Goodness of fit AUC=0.856; McFaddens R^2 = 0.320; goodness of link ascertained via linktest in Stata.
Base refers to the reference category in the analyses.

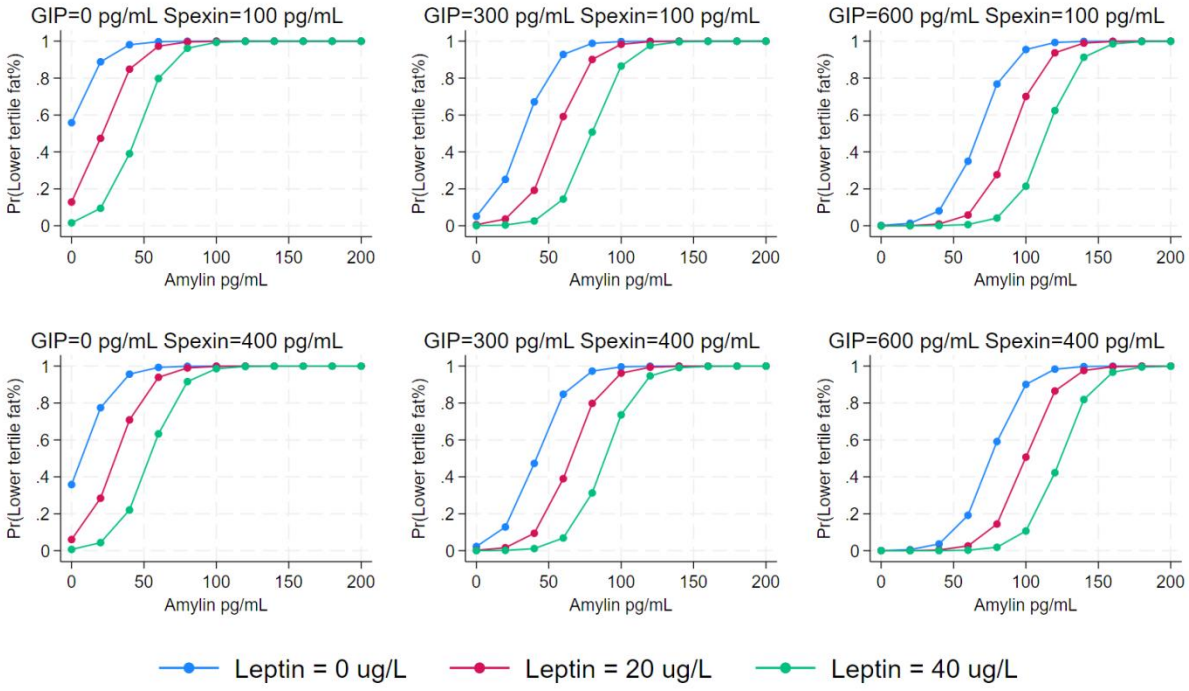

Figure 1. Margins plot with the probability of having low fat% (lower tertile) as a function of serum Amylin (pg/ml) level across two Spexin (pg/ml) levels (100 and 400), three GIP (pg/ml) levels (0, 300, 600) and three Leptin (ug/L) levels (0, 20, 40).

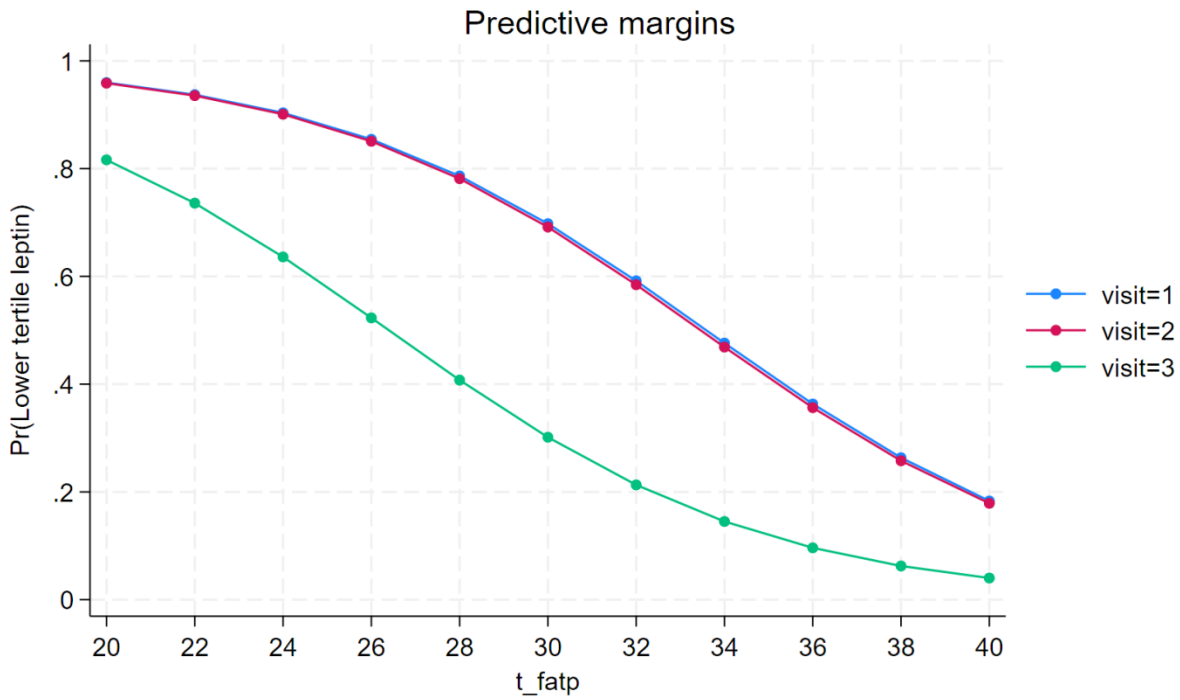

Figure 2: Margins plot with the probability of having low Leptin (ug/L) levels (lower tertile) as a function of fat % across the three visits.

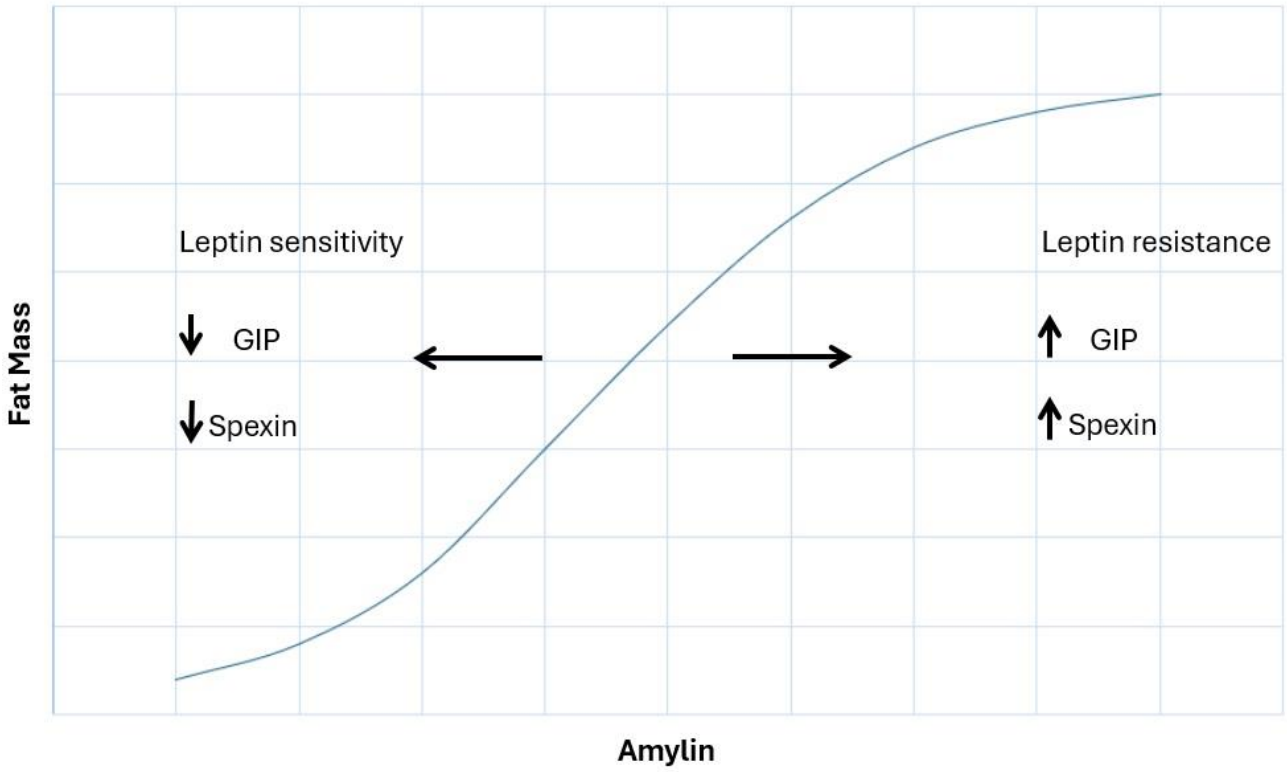

Figure 3. This diagram illustrates the relationship between Amylin levels and Fat Mass, highlighting key factors that modulate this relationship based on our findings.