Spexin Is a Novel Human Peptide that Reduces Adipocyte Uptake of Long Chain Fatty Acids and Causes Weight Loss in Rodents with Diet-Induced Obesity

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Objective: Microarray studies identified Ch12:orf39 (Spexin) as the most down-regulated gene in obese human fat. Therefore, we examined its role in obesity pathogenesis.

Methods: Spexin effects on food intake, meal patterns, body weight, respiratory exchange ratio (RER), and locomotor activity were monitored electronically in C57BL/6J mice or Wistar rats with diet-induced obesity (DIO). Its effects on adipocyte [³H]-oleate uptake were determined.

Results: In humans, Spexin gene expression was down-regulated 14.9-fold in obese omental and subcutaneous fat. Circulating Spexin changed in parallel, correlating (r = 0.797) with Leptin. In rats, Spexin (35 µg/kg/day SC) reduced caloric intake 32% with corresponding weight loss. Meal patterns were unaffected. In mice, Spexin (25 µg/kg/day IP) significantly reduced the RER at night, and increased locomotion. Spexin incubation in vitro significantly inhibited facilitated fatty acid (FA) uptake into DIO mouse adipocytes. Conditioned taste aversion testing (70 µg/kg/day IP) demonstrated no aversive Spexin effects.

Conclusions: Spexin gene expression is markedly down-regulated in obese human fat. The peptide produces weight loss in DIO rodents. Its effects on appetite and energy regulation are presumably central; those on adipocyte FA uptake appear direct and peripheral. Spexin is a novel hormone involved in weight regulation, with potential for obesity therapy.

Introduction

The worldwide epidemic of obesity is expected to remain one of the greatest challenges to public health in the 21st century. The United States in particular is experiencing an obesity epidemic with profound consequences (1), for example, ~300,000 deaths annually (2), a decrease in life expectancy (3), and enormous health care costs (4). Obesity is linked to well documented increases in the prevalence of many conditions that cause excess morbidity and mortality (5).

White adipose tissue (WAT) plays a major role in energy storage. In addition, distinct WAT depots also function as “endocrine organs” by secreting unique profiles of adipokines, a diverse collection of more than 50 cytokines, chemokines, and hormone-like factors, which contribute to the maintenance of energy homeostasis. Although not expressed exclusively by WATs, some locally secreted adipokines have been shown to affect appetite, satiety, and glucose and lipid metabolism (6). The actions of many of these adipokines are ultimately integrated to regulate glucose and energy metabolism, long chain fatty acid (LCFA) uptake and storage, and insulin activity via both paracrine and endocrine mechanisms.

Since 2008, microarray studies in our laboratory comparing gene expression in obese versus nonobese human omental and abdominal subcutaneous fat identified Ch12:orf39 (Spexin) as the most down-regulated gene in obese human fat. Therefore, we examined its role in obesity pathogenesis.
subcutaneous fat have identified both individual genes and biological pathways whose components are significantly dysregulated in obese fat. Among our earliest studies, using arrays with probes for ~55K genes and expressed sequence tags (ESTs), we identified ~3,500 genes and ESTs that exhibited significant differences in expression (7). Of these, the most down-regulated gene was Ch12orf39, whose mRNA was under-expressed 14.9-fold in obese fat. It appeared to encode a secreted peptide, which we subsequently recognized was identical to Spexin, a novel peptide identified by Mirabeau et al. in 2007 using Markov modeling (8).

That Spexin was the single most down-regulated gene in obese human fat, coupled with observations by Mirabeau et al. that Spexin induced muscarinic-like contractions in stomach smooth muscle in vitro (8), led us to postulate that it might normally function as an adipocyte-expressed satiety factor, and that the lack of Spexin expression by obese fat might lead to the loss of a key adipokine potentially involved in the regulation of gut motility, food intake, energy metabolism, and LCFA uptake and storage in adipocytes. Therefore, we set out to define its biological role in rodent models of diet-induced obesity (DIO).

Methods

Patients

Patients undergoing clinically indicated abdominal laparoscopic surgical procedures consented to removal of omental and subcutaneous fat samples for studies of LCFA transport, molecular studies, and a venous blood sample for the measurement of circulating adipokines. Obese patients were undergoing bariatric surgical procedures, and the nonobese patients were undergoing other clinically indicated laparoscopic procedures at either the Weill Cornell or Columbia Presbyterian campuses of New York Presbyterian Hospital. The protocols, consent documents, and procedures for these studies were approved by the individual Institutional Review Boards of the Columbia University and Weill Cornell Medical Centers.

Materials

9,10-[^3]H-Oleic acid (OA) was purchased from NEN Life Science Products (Boston, MA), type I collagenase from Sigma (St. Louis, MO), and FA free bovine serum albumin (BSA) from Boehringer Mannheim (Indianapolis, IN).

Two preparations of Spexin were used: the first from Phoenix Pharmaceuticals (Burlingame, CA) and the second, a custom synthesis product from the Ferring Research Institute (San Diego, CA). Both were >95% pure by HPLC and ID’d by LC/MS.

Isolation of adipocytes

Adipocytes from human omental and subcutaneous fat biopsies (9) and from epididymal fat pads of obese mice (18 weeks of age) (10) were isolated and sized by direct light microscopy as described (9,10).

Studies of LCFA uptake kinetics

The initial rate of[^3]H-OA uptake by both human and mouse adipocytes was determined by rapid filtration (9,11). The unbound oleate concentration ([OAU]) in each test solution was calculated from the OA/BSA molar ratio (v) (12), using the LCFA/BSA binding constants of Spector et al. (13). Data fitting used the SAAM II program of Berman and Weiss (14).

Statistical considerations

Values for physiologic variables, unless otherwise noted, are reported as the mean ± standard error, and calculated according to standard methods of descriptive statistics (15). The significance of differences between groups was assessed with Student’s two-tailed t-tests, with P ≤ 0.05 being considered significant.

Gene expression studies

Whole human genome microarray. Gene expression in omental and subcutaneous fat samples from obese versus nonobese subjects was compared by whole genome microarray analysis as previously described in detail (7). Median normalized gene expression data (arbitrary expression units) were analyzed using the GeneSifter Data package, with statistical treatment of the results as reported earlier (7).

qRT-PCR. Spexin gene expression was examined in the same adipose tissue samples and additional obese and nonobese human omental adipose tissues by qRT-PCR according to the methods reported earlier (7). Spexin primers for PCR were designed using Primer 3 software (v.0.4.0) at http://fokker.wi.mit.edu/primer3/input.htm. Similar methods were used to test for Spexin gene expression in mouse epididymal adipose tissues. Specific primer sequences for both human and mouse Spexin are presented in Supporting Information.

Quantitative immunoassays

Quantitative immunoassays to determine circulating levels of Spexin by competitive enzyme immunoassay (EIA) (Cat # EK-023-81) and human Leptin by antigen capture ELISA (Cat # EK-003-12) were performed with kits purchased from Phoenix Pharmaceuticals (Burlingame, CA). Unknown samples were diluted 1/20 in assay buffer, and measured OD values were quantified by comparison to within-assay standard curves. Quantitative validation was also confirmed by “spike-in” experiments, in which known amounts of Spexin standard were added to individual serum samples (see Supporting Information).

Metabolic assessments in DIO mice

Metabolic and behavioral measurements were performed as previously described (16). Individually housed C57BL/6J mice with DIO or age-matched controls (Jackson Labs, Bar Harbor, ME) were maintained ad lib on a high fat diet (HFD, D12492, Research Diets, New Brunswick, NJ) which provided 60% of total calories as fat.

24-h feeding behavior in DIO rats

Obese (DIO) adult female Wistar rats were maintained ad lib on 60% HFD (D12492) in individual chambers in which their feeding behavior was continuously recorded via a BioDaq Electronic Food Intake Monitoring System (Research Diets, New Brunswick, NJ).

Conditioned taste aversion studies in rats

Conditioned taste aversion studies were conducted using adult female Wistar rats according to established protocols (17-19). For training,
rats were water, but not food, deprived for 20 h (18:00-14:00 h the following day), then offered a single bottle of water ad libitum for 20 min. Training took place on 2 days of each week, spaced at 3-4 day intervals, to allow recovery of normal food intake and body weight. A total of seven training sessions were conducted, by which time all animals had learned to drink promptly when fluid was offered.
Results

Gene expression profiling of human fat

Gene expression in omental and subcutaneous fat samples from obese versus normal weight subjects was compared by whole genome microarray analysis (7). Median normalized gene expression ratios for each probe, representing 55K known genes and ESTs, were compared by log–log plot (Figure 1A). The mRNA for Ch12, ORF39 (Spexin) demonstrated a highly significant 14.9-fold drop in expression in obese compared to nonobese fat tissues (Figure 1B), the largest change in gene expression observed in obese human fat ($P < 0.00292$).

Validation of gene expression results by qRT-PCR. Observed changes in Spexin gene expression were validated by qRT-PCR of all of the original fat samples assayed initially by microarray, another 12 omental samples from obese patients (final $n = 18$) and four from additional nonobese tissue donors (final $n = 9$). When assayed by qRT-PCR, Spexin mRNA demonstrated a 33.3-fold decrease in Spexin gene expression in human obese, compared to nonobese fat samples (Figure 1C; $P < 0.001$).

Spexin gene expression was also analyzed in 20-week-old DIO mice ($n = 10$) and C57BL/6J controls ($n = 8$) by qRT-PCR. The normalized Spexin gene expression ratio in the DIO mice was $0.638 \pm 0.107$ compared to $1.00 \pm 0.091$ for the normal weight controls ($P = 0.047$) (Supporting Information, Figure 1A).

Spexin and Leptin concentrations in serum

Sera from nonobese and obese patients (BMIs $= 23.5 \pm 0.9$ vs. $49.3 \pm 1.8$, respectively) were assayed for circulating Spexin and Leptin concentrations by commercial immunoassays. The mean
concentration of Spexin peptide in the serum of obese patients was approximately 10% of that in nonobese subjects (1.1 ± 0.7 vs. 11.6 ± 1.3 ng/mL; obese vs. nonobese; n = 7/group; P < 0.0002), in agreement with the 15-fold reduction in Spexin gene expression found in WATs from obese patients. Circulating Leptin averaged 8.5 ± 3.5 ng/mL in serum from nonobese patients, and 37.4 ± 4.7 ng/mL in serum from obese patients (P = 0.014). There was a highly significant, nonlinear, negative correlation r = −0.797, P < 0.01) between Spexin and Leptin concentrations in human sera (Figure 2A). In addition, Spexin concentrations were significantly negatively correlated (Figure 2B) and Leptin concentrations positively correlated (Figure 2C) with the V_{max} for LCFA uptake by omental adipocytes from the same patients. Circulating Spexin concentrations were also measured in 20-week-old DIO mice (2.24 ± 0.07 ng/mL, n = 17) and age-matched C57BL/6j background controls (4.28 ± 0.11 ng/mL, n = 5) (Supporting Information Figure 1B). As in the human samples, circulating Spexin concentrations were significantly higher in the nonobese control mice than in the obese DIO animals (P < 0.001).

The less dramatic differences in Spexin expression and serum levels in the DIO mice may reflect either an intrinsic species-specific difference, or that the DIO mice weighed only approximately 1.5× as much as the nonobese animals, while the obese patients in this study weighed 2.5 times as much as the nonobese study participants.

Effect of exogenous Spexin on body weight in C57BL/6j mice with DIO

To test our hypothesis that Spexin is a satiety factor that modulates feeding behavior, we administered various Spexin doses to DIO mice by daily IP injection for up to 50 days. Control animals received daily IP injections of equal volumes of 1× phosphate-buffered saline (PBS). For these studies, mice were housed five to a cage in standard plastic box cages. In addition to body weights, food and water consumption were measured daily in some of these studies. Spexin treatment consistently resulted in weight loss. In a representative study, animals treated with Spexin at 25 μg/kg (IP QD) lost weight over the course of the experiment, while controls receiving 1× PBS continued to gain weight on the HFD (Figure 3). In parallel studies, in which food consumption was also measured, food consumption declined progressively over time in Spexin-treated mice, but remained essentially unchanged in PBS-treated controls (Supporting Information Figure 2). By contrast to the obese DIO mice, normal weight C57BL/6j mice did not lose weight when treated with similar doses of Spexin.

Effects of exogenous Spexin on metabolic parameters in DIO Mice

In a subsequent study, after adaptation to metabolic cages, individually housed mice were treated daily either with Spexin (35 μg/kg IP) or an equivalent volume of PBS for 19 days. Body weights were recorded daily. As indicated by the slopes and correlation coefficients of the weight versus time regression lines, the vehicle treated animals continued to gain weight over the course of the experiment (y = + 0.1625x + 39.801, r = +0.9262), while the Spexin-treated animals lost weight over the same period (y = −0.0666x + 38.3, r = −0.7318) (Figure 4A). All animals were monitored with measurements of O$_2$ consumption, CO$_2$ production, the respiratory exchange ratio (RER), and energy expenditure (EE) throughout the study (Figure 4B through 4E). Metabolic results such as these may be expressed either per mouse or per gram BW. They are displayed in Figure 4 on a per mouse basis. However, neither VO$_2$ nor EE were significantly affected in Spexin-treated mice, regardless of whether the data are expressed per mouse or per gram BW. Tracings of ambulatory events counts and the RER across representative 24-h periods during this study are presented in Figure 5. Unfortunately, efforts to monitor the sizes of individual meals and cumulative food intake electronically during this study were unsuccessful.

Spexin inhibits LCFA uptake into isolated adipocytes

Multiple studies suggest that regulation of adipocyte LCFA uptake is an important control point for body adiposity, for example, Refs. 9-11,24). To study the potential role of Spexin in this process, epididymal fat pads were removed from Spexin-treated DIO mice, and [3H]-oleic acid uptake kinetic constants in isolated adipocytes were determined (9-11,25). The V_{max} for facilitated LCFA uptake in Spexin-treated mice, 61 ± 9 pmol/s/50,000 cells, was only 28% of that of the control mice (211 ± 60 pmol/s/50,000 cells, P = 0.047). Adipocyte suspensions were also prepared from untreated DIO mice. Paired aliquots were incubated with either PBS or PBS/Spexin (20 ng/mL). Short incubations in vitro (2 h) resulted in approximately a 40% down-regulation of adipocyte LCFA uptake (data not shown). Nearly identical results were obtained with both Spexin preparations tested (Phoenix Pharmaceuticals, Ferring Research Institute). Finally, adipocytes isolated from untreated DIO mice were incubated for 2 h in either PBS alone or PBS + Spexin at one of ten concentrations from 0.01 to 80 ng/mL. LCFA uptake kinetics were then determined. A total of 38 triplicate uptake inhibition studies were performed (Figure 6). They reveal a bimodal response of LCFA uptake inhibition to the concentration of Spexin employed, with maximal inhibition of 73 ± 6% of control LCFA uptake at a Spexin concentration of 1 ng/mL.

Effect of Spexin on 24-h feeding behavior and body weight in DIO rats

Female DIO rats used in this study were approximately 20% overweight for age at the beginning of treatment. Individual body
weights and total food consumed were measured daily. Reductions of daily food intake seen by Day 3 (∼32%) were statistically significant on Days 4-6, the final treatment day, and the first two washout days, (Figure 7A). The reduction in body weight noted by Day 4 persisted well beyond the treatment period (Figure 7B). Cumulative 24-h food intake curves from this study were obtained via

**Figure 4** Effects of Spexin on metabolic parameters in DIO mice. After adaptation, 18–20-week-old DIO mice were dosed with Spexin (35 μg/kg IP in 1× PBS) daily for 19 days while individually housed in metabolic chambers. Individual body weights, 24-h locomotor activity (ambulation in the X, Y, and Z planes), oxygen consumption, CO₂ output, and the RER were monitored continuously using a CLAMS (Columbus Instruments, Columbus, OH) open-circuit indirect calorimetry system (16,23). As in earlier studies (e.g., Figure 3), Spexin-treated animals lost weight but control mice continued to gain weight on the HFD (A). Metabolic parameters such as VO₂, VCO₂, and EE may be expressed on either a per mouse or per gram BW basis. We have displayed them in Figure 4 on a per mouse basis. However, neither VO₂ nor EE were significantly affected in Spexin-treated mice regardless of whether the data are expressed per mouse or per gram BW. Oxygen consumption (VO₂) (B) was modestly increased during the dark cycle (lights off), when the mice are more active, in both control and Spexin-treated animals, compared to the light cycle. Overall, the Spexin-treated mice demonstrated a very modest, nonsignificantly lower VO₂ through the light, dark, and total periods compared to that in control animals. Carbon dioxide production (VCO₂) during the light and dark cycles (C) exhibited a similar pattern to that of VO₂, being somewhat lower in Spexin-treated versus control mice. The cumulative RER results over 24 h (D), which represent the ratio of VCO₂/VO₂, show different patterns between the light (12 h) and dark (12 h) periods, and between the Spexin treated and control mice. The control mice exhibited an expected, modest elevation in RER during the dark compared to the light cycle, whereas that in the Spexin-treated animals changes very little during the dark cycle. Consequently, the Spexin-treated animals have significantly lower RERs compared to the control mice during the dark cycle (0.784 ± 0.002 vs. 0.795 ± 0.002, P = 0.006, respectively). The 24-h total RERs were also lower in the Spexin-treated animals, although this difference did not quite achieve statistical significance (P = 0.068) (Figure: * = P < 0.05, ‡ = 0.10 > P > 0.05). Total EE showed little difference in any comparison (E). The total kcal/mouse/h expended increased slightly during the dark versus the light periods in both groups, however, the total amounts of energy consumed during each period were almost identical between the Spexin-treated animals and the controls.
At baseline, the two groups of animals demonstrated essentially identical feeding behavior. It is noteworthy that meal frequency and the temporal (light/dark) feeding pattern during the 24-h feeding periods remained normal in the Spexin-treated group throughout the treatment and washout periods. However, both meal size (grams of food consumed/meal) and meal duration (time spent feeding/meal) were smaller in the Spexin-treated animals, who consumed approximately 32% fewer calories overall than controls during the last day of treatment and the first two days following treatment.

**Conditioned taste aversion testing in Wistar Rats**

The total amount of saccharin solution consumed by the LiCl-treated animals was significantly less than that consumed by either the saline or Spexin-treated animals (Figure 8, \( P < 0.01 \) or smaller). This was noted by Day 2 of testing, and persisted throughout the challenge period. No significant differences in saccharin consumption from the vehicle-injected group were noted in the Spexin-treated animals, indicating that reductions in 24-h food consumption previously seen in Spexin-treated animals were not due to aversive effects of Spexin on ingestive behavior.

**Discussion**

Spexin was first identified as a novel peptide hormone by Mirabeau et al. (2007), using an approach based on hidden Markov model screening to identify novel peptide-encoding sequences in the human genome (8). Virtually nothing was known initially about its biological activity. Microarray studies on surgical fat biopsies in our laboratory starting in 2008 identified Ch12:orf39 as the gene with the greatest difference in expression between obese and nonobese...
human fat. We subsequently recognized that Ch12:orf39 encoded Spexin, the peptide identified by Mirabeau et al. (8).

Our finding that Spexin was the most down-regulated gene in microarray studies in obese human fat (7), and the demonstration of its contractile activity in an in vitro rat stomach explant model system (8), led us to postulate that Spexin might function as an adipocyte-expressed satiety factor. We speculated that the lack of Spexin expression by obese fat might reflect the loss of a key adipokine, which would potentially impact the regulation of gut activity, food consumption, energy metabolism, and LCFA uptake and storage in adipocytes. A commercial immunoassay allowed us to examine possible relationships between circulating levels of Spexin and those of known obesity-related adipokines in human sera.

Leptin is known to play a major role in the regulation of body weight and food consumption (10,11,26), and its expression is elevated in obesity (26). The strong negative correlation (see Figure 2A) between Leptin and Spexin in the serum of obese patients and normal weight controls supports the idea that these peptides might play antagonistic roles in the normal regulation of hunger, satiety, body adiposity and weight by serving as opposing components of a negative feedback loop. We, therefore, explored the biological role of Spexin in energy metabolism and storage in two rodent models of DIO. Daily intraperitoneal injections of Spexin led to a reduction in food consumption and body weight in DIO mice, while vehicle-injected DIO mice continued to gain weight. Analogous data were obtained in a separate study with DIO rats. The lack of detectable taste-aversive effects of Spexin in formal testing appreciably enhances the importance of these observations.

Figure 7 Daily injections of Spexin into DIO rats lead to reductions in food intake (A), body weight (B), and size and duration of individual meals (C–F). Female DIO rats received single daily injections (SQ) of vehicle (PBS) or Spexin (n = 4/group) over a 4-day period, with Spexin doses set at 35 µg/kg BW for the first 3 days and 70µg/kg BW on the fourth day. Body weight and 24-h food intake of the rats were recorded daily during the treatment period, and for 11 consecutive posttreatment days (‘washout’ days). (A) Significant reductions (ca. 32%) of daily food intake were seen on Days 4–6 of the experiment (the final treatment day and first two washout days) that persisted at least to Day 7 (*P < 0.05, **P < 0.01 vs. PBS controls). (B) A reduction in body weight was apparent by Day 4 and persisted well beyond the treatment period. An analysis of the number of feeding events or ‘meals’ during each 24-h light/dark cycle, and the size and duration of individual meals was conducted using computer-recorded feeding data. Individual body weights and total food consumed were measured daily. Thicker horizontal black lines at the base of the figures indicate the ‘lights on’ period, with the ‘lights off’ period running from 19.30 to 7:30 h. As expected, the vast majority of feeding occurs during ‘lights off.” (C) Baseline: When the lights are turned off (dark period), both groups of DIO rats begin to consume food at a relatively steady rate. Meal pattern analysis revealed that meal frequency, meal duration, and meal size were very comparable between the two groups at this point. (D) On-treatment: By the fourth treatment day Spexin-treated animals (70 µg/kg) demonstrated reduced meal size and duration, without altering their overall feeding pattern. Food consumption was lower during the normal feeding period (lights out; 19:00-07:00 h), and the animals maintained the normal cessation of feeding behaviors during the ‘lights on’ period. No signs of overt toxicity or nausea were observed in the Spexin-treated rats. (E) Day 1 wash-out: during the initial day of post-Spexin treatment, the satiety effect of the peptide begins to diminish. (F) Day 3 wash-out: several days after the last injection of Spexin, both groups of animals again demonstrate equivalent total 24-h food consumption and meal patterns. It is noteworthy that meal frequency and the temporal (light/dark) feeding pattern during the 24-h feeding periods remained normal in the peptide-treated group throughout the treatment and washout periods. However, both meal size (grams of food consumed/meal) and meal duration (time spent feeding/meal) were smaller in the Spexin-treated animals, who consumed approximately 32% fewer calories overall during the last day of treatment and the first 2 days following treatment. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Spexin-treated animals showed a significant decrease in food intake compared to LiCl-treated animals on the second injection day. The results served as a positive control arm (for taste aversion) with which to assess the effects of repeated injections of Spexin. The average amount of saccharin solution consumed during each 20 min test period was recorded for each group. A mild dose of LiCl was used over repeated test sessions to generate a slowly developing conditioned taste aversion. LiCl results served as a positive control arm (for taste aversion) with which to assess the effects of repeated injections of Spexin. The total amount of saccharin solution consumed during the 20 min test by the LiCl-treated animals on the second injection day and following was significantly less than consumed by either the saline or Spexin-treated animals, **P < 0.01, ***P < 0.001. Data points represent mean ± SEM. No significant difference in the volumes of saccharin solution consumed were observed in the Spexin-treated animals.

These findings were further pursued in separate cohorts of mice undergoing Spexin treatment for 19 days while in metabolic chambers. The calorimetry cage studies revealed that Spexin significantly reduced the RER, suggesting preferential fat oxidation, particularly during darkness, when the difference in RER between Spexin- and vehicle-treated mice was highly significant. The observed reduction in the RER at night appears to result from inhibition of the normal nighttime elevation in carbohydrate metabolism, with an attendant shift to lipid oxidation. Spexin also significantly increased locomotor activity, but only in the Z-plane, by “rearing.” There was no evidence of randomly increased “anxiety” activity.

The basis for the reduction in RER and the increase in locomotor activity in Spexin-treated animals remains speculative. Nevertheless, the reduction of food intake without taste aversion, altered metabolism, and increased locomotor activity during Spexin administration suggest that these effects collectively may be centrally mediated. This question will be pursued going forward by studying the effects of Spexin administration in positron emission tomographic scanning of brain activity, and of the effects of low dose intracerebroventricular Spexin administration on food consumption and body weight.

The regulation of facilitated LCFA uptake into adipocytes has been proposed to be an important control point for body adiposity (9). This process has been studied extensively in our laboratory using dietary manipulations in intact rodents, and in adipocytes isolated from various genetic and diet-induced animal models of obesity and from obese patients (9-12,24,25). To test the potential role of Spexin in the regulation of LCFA uptake into adipocytes, we first showed that Spexin treatment of DIO mice in vivo significantly inhibited facilitated LCFA uptake into adipocytes freshly isolated from the treated animals. We subsequently studied the pharmacodynamics of acute in vitro Spexin incubation on adipocytes freshly isolated from untreated DIO mice. The resulting concentration-response curve for Spexin-induced inhibition of LCFA uptake into adipocytes was biphasic, demonstrating a maximum 73% inhibition of LCFA uptake at a Spexin concentration of 1 ng/mL. These data clearly indicate that, in addition to effects that likely are centrally mediated, Spexin has direct effects on peripheral adipocytes. Such a curve is an example of “hormesis,” a phenomenon which is typically reflected in a biphasic peptide concentration-response curve (27-29). It is most often seen with peptide hormones whose effects are receptor-mediated, when the peptide is tested at doses that span several orders of magnitude (27,30). These results are consistent with the hypothesis that Spexin may play a role in the regulation of LCFA uptake into adipocytes via a receptor-mediated process.

Since the original report (8) seven more articles about Spexin have appeared (31-37), one of which identified the same gene sequences, designated NPQ, using an alternative bioinformatics approach (35). In rats, low level Spexin expression was detected by RT-PCR in all tissues studied, including esophagus, stomach, small intestine, liver, pancreas, lung, skeletal muscle, heart, uterus, thymus, spleen kidney, bladder, specific brain regions, and multiple endocrine organs including anterior pituitary, adrenal, thyroid, testis, and ovary. Spexin was also widely identified in rat tissues by immunohistochemistry (32). However, its expression in fat has not previously been described. It has been reported to have a number of endocrine functions (31,33,36) and to be a modulator of cardiovascular and renal function (36), although in the latter case the doses administered by rapid IV bolus injection to achieve the transient effects reported were very large and certainly supraphysiologic (up to 10× what we administered daily intraperitoneally to produce weight loss). After this article was initially submitted, two studies in goldfish (Carassius auratus) were published, demonstrating (A) that injection of goldfish Spexin into the CNS led to a decrease in feeding behaviors and total food consumption through selective alterations in the expression of orexigenic and anorexigenic signals in the telencephalon, optic tectum, and hypothalamus (37), and (B) that Spexin specifically suppressed luteinizing hormone release (31). Thus, like Leptin, Spexin appears to be an adipokine with not only major roles in the regulation of food intake and body weight but also diverse endocrine effects.

To our knowledge, we are the first to demonstrate Spexin expression in human WAT, to identify the almost complete absence of Spexin expression in human obese WAT, to propose that Spexin has a role in the normal regulation of adipose tissue function including uptake of LCFA, that the absence of Spexin may be a major component of the hormonal dysregulation seen in obese fat, and that repletion of circulating Spexin may help restore normal feeding behaviors and energy balance in obese animals and man. The Spexin story is clearly just beginning and there are many unanswered questions, of which the effects of Spexin on body composition is an important one, but what is reported here clearly moves the field forward.

This study, one result of a productive collaboration between a laboratory focused on lipid metabolism and a team of bariatric surgeons, strongly supports the hypothesis that Spexin is a potent, natural satiety-inducing peptide that plays a key role in regulating feeding...
behavior, uptake of LCFAs into adipocytes, energy utilization and metabolism, and body weight in DIO mice and rats. Its therapeutic potential for the treatment of human obesity merits detailed exploration.

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