Rational Design of a Combination Medication for the Treatment of Obesity

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Existing obesity therapies are limited by safety concerns and modest efficacy reflecting a weight loss plateau. Here, we explore combination therapy with bupropion (BUP), a putative stimulator of melanocortin pathways, and an opioid antagonist, naltrexone (NAL), to antagonize an inhibitory feedback loop that limits sustained weight reduction. *In vitro* electrophysiologic experiments were conducted to determine the extent to which BUP+NAL stimulated hypothalamic pro-opiomelanocortin (POMC) neurons in mouse brain. A subsequent study further characterized the effect of combination BUP+NAL treatment on food intake in lean and obese mice. Finally, a randomized, blinded, placebo-controlled trial in obese adult subjects was conducted. Randomization included: BUP (300 mg) + NAL (50 mg), BUP (300 mg) + placebo (P), NAL (50 mg) + P or P+P for up to 24 weeks. BUP+NAL stimulated murine POMC neurons *in vitro* and caused a greater reduction in acute food intake than either monotherapy, an effect consistent with synergism. Combined BUP+NAL provided sustained weight loss without evidence of an efficacy plateau through 24 weeks of treatment. BUP+NAL completers diverged from NAL+P ($P < 0.01$) and P+P ($P < 0.001$) at week 16 and from BUP+P by week 24 ($P < 0.05$). The combination was also well tolerated. Translational studies indicated that BUP+NAL therapy produced synergistic weight loss which exceeded either BUP or NAL alone. These results supported the hypothesis that NAL, through blockade of β-endorphin mediated POMC autoinhibition, prevents the classic weight loss plateau observed with monotherapies such as BUP. This novel treatment approach (BUP+NAL) holds promise for the treatment of obesity.

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INTRODUCTION

One-third of the adult US population is obese and another third is overweight, as defined by BMI (1). Because obesity is associated with numerous medical complications including type 2 diabetes, cardiovascular disease, hypertension, depression, coronary artery disease, stroke, and some cancers, it presents a serious health problem (2). Obesity-related morbidity leads to increased use of hospitalization and greater mortality than normal weight adults (3), making obesity second only to smoking as the leading cause of preventable death (4). In the United States, obesity accounts for several hundred thousand deaths annually and current obesity-related medical costs are estimated at more than $100 billion per year (5). Despite dramatic advances in our understanding of central regulation of energy balance and obesity, there are few approved obesity pharmacological treatments, and their use has been limited by their modest efficacy and/or safety concerns.

Pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus (ARH; also called the infundibular nucleus) integrate central and peripheral signals related to energy balance and produce a net anorexigenic output (6). The well-publicized anorectic effect of the hormone leptin is primarily due to stimulation of POMC cells (6,7). POMC cells can become leptin resistant in obesity which may lead to decreased basal activity and a lower anorexigenic drive in spite of increased adiposity (8,9). Therefore, agents that stimulate POMC signaling are of interest in obesity pharmacotherapy, however efforts employing direct melanocortin agonists are associated with side effects (10) and current clinical experience with monotherapeutic drug approaches has been only modest

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weight loss which appears to be limited by a therapeutic plateau e.g., bupropion (BUP) (11). One hypothesis for this limited efficacy is that POMC stimulation results in compensatory autoinhibitory feedback. In the melanocortin system, activity of POMC neurons is regulated by endogenous opioids such as β-endorphin, a product of POMC cleavage (6). We and others have shown that POMC neurons are inhibited by opioids (7,12,13). Consequently, we have hypothesized that agents that suppress this inhibitory opioid feedback may facilitate greater weight loss when used as an adjunct to a POMC stimulator.

Combination therapies are often used to treat chronic diseases, including common conditions such as diabetes and hypertension (14,15), and are especially attractive for pathologic states where multiple regulatory mechanisms may contribute to the development or maintenance of disease. An advantage of combination therapies is the potential for additive, complementary, or even synergistic effects. To date, limited attempts at combination therapies for obesity have failed to demonstrate a desired benefit:risk profile. The combination of fenfluramine and phentermine (fen-phen) was associated with significant weight loss that exceeded the effects of either agent alone (16) but fenfluramine was withdrawn from the market for safety reasons (17). Clinical trials with the combination of sibutramine and orlistat failed to demonstrate even additive weight loss (18).

BUP is a dopamine and norepinephrine reuptake inhibitor well known for its role in the management of depression and smoking cessation (19,20). The effect of BUP as a weight-loss therapy in obese adults (11) is probably due to a combined dopaminergic and noradrenergic effect on POMC signaling (21–24), however weight loss benefits have been modest and plateau within several months of therapy. Naltrexone (NAL) is an opioid receptor antagonist, initially approved as a treatment for opioid dependency, and later for alcohol dependence (25). As a monotherapy, NAL is associated with minimal weight loss (26,27). Thus, one would not necessarily predict that combining these two agents would be associated with a clinically meaningful and sustained pattern of weight reduction. However, we hypothesized that the addition of NAL would antagonize the normal inhibitory feedback mechanism that limits sustained POMC activation in response to agents such as BUP and contribute to an improved therapeutic profile. To test this hypothesis, we first conducted a series of preclinical studies to explore the effects of combination therapy with BUP+NAL on energy balance. We began by confirming *in vitro* the cellular mechanism for increased firing of POMC neurons with BUP+NAL. We then demonstrated in a relevant animal model that coadministration of BUP+NAL caused greater reductions in food intake in lean and obese mice than was seen with the constituents administered individually. We concluded this sequence of translational studies with a proof-of-concept study in human obesity.

METHODS AND PROCEDURES

Preclinical studies

Electrophysiological recording from mouse POMC neurons. *In vitro* stimulation of POMC neurons in the ARH is associated with decreased food intake and increased energy expenditure (6). Therefore, the effect of BUP, NAL, and combined BUP+NAL on the firing frequency of POMC neurons was investigated. Whole-cell and loose-patch recordings were obtained from identified POMC neurons in hypothalamic slices from POMC-enhanced green fluorescent protein mice (POMC–EGFP mice). Electrophysiological recordings and generation of the transgenic mice was performed as described previously (7). Briefly, coronal hypothalamic slices containing the ARH were cut at 185 µm and maintained and recorded from at 35 °C in artificial cerebrospinal fluid (aCSF; in mmol/l: 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 21.4 $NaHCO_3$, 5.0 D-glucose, 5.0 D-mannitol). Slices were saturated with 95% O_2/5% CO_2 for 1 h before recordings. Whole-cell recordings were made from fluorescent neurons using an Axopatch 200B patch-clamp amplifier and analyzed using pClamp 8.0 (Axon Instruments, Foster City, CA) and MiniAnalysis 5.0 (Synaptosoft, Decatur GA). Current clamp recordings were performed utilizing an intracellular solution of the following composition (mmol/l): 128 K gluconate, 1 EGTA, 10 KCl, 1 $MgCl_2$, 0.3 $CaCl_2$, 5 ATP, 0.3 GTP, 10 HEPES; pH 7.3. For cell-attached patch, the pipettes were filled with aCSF. Drugs were bath applied for about 550 s by a gravity-fed system; it is expected that tissue bath drug equilibration occurs 45 s following the initiation of drug application. The extracellular technique that we use here does not allow us to record any membrane properties, but only frequency of action currents. We determined effects of drug vs. vehicle and whether the effect fades as the drug is slowly washed from the bath. Only cells demonstrating a reversible drug effect are presented.

Animal care and housing. All animal procedures were approved by the Oregon National Primate Research Center Institutional Animal Care and Use Committee. Adult male C57Bl/6J mice (Jackson Labs, Bar Harbor, ME) were individually housed under a 12 h light/dark cycle. Food and water were available ad libitum, unless specified otherwise. Mice were maintained on standard chow except where noted (Purina Lab Chow no. 5001; 3.34 kcal/g of energy, 59.8% carbohydrate, 28.0% protein, and 12.1% fat).

Generation of obese (DIO) mice. The group of 8 week-old male mice was kept on a high-fat diet (Rodent Chow no. D12451, Research Diets, New Brunswick, NJ; 4.75 kcal/g of energy, 35.0% carbohydrate, 20.0% protein, and 45.0% fat) for 20 weeks. After 20 weeks, DIO (diet-induced obese) mice were characterized by increased body weight and fat deposits, and reduced central leptin sensitivity (9,28). Lean age-matched mice (control) were also put on a high-fat diet just prior to feeding tests. The novel high-fat diet causes a temporary increase in food intake so feeding tests began when daily food intake stabilized (about 10 days). All behavioral studies in obese mice (and lean control) were conducted by measuring intake of high-fat diet.

Feeding studies. Individually housed mice were habituated to the behavioral testing procedure by one or two IP injections of 0.1 ml sterile saline daily and 16 h (overnight) fasting every third day for at least 1 week before treatment. Animals were randomly distributed (according to body weight) to treatment groups. On the morning of the testing day, fasted mice were injected with drug or vehicle (13.4% ethanol, 20.1% propylene glycol, 66.5% saline), and given previously weighed food. Food was weighed at 1, 2, 4, 8, and 24 h postinjection. For initial dose–response studies drug doses were based on effective doses in mice, as extrapolated from the literature and previous experience in our laboratory. Initial dose–response studies were designed to include a broad range of doses spanning 1.5–2 units on a log scale. The purpose of dose–response studies was twofold. First, dose–response studies demonstrated that acute BUP or NAL administration consistently and dose-dependently reduces food intake in fasted mice. Second, dose–response curves provided an estimate of hypophagic efficacy from which future drug doses could be selected. Animals were closely monitored by the investigator following drug injection and any adverse side effects were noted. For example, high doses of BUP can produce

seizures (23); however the highest dose of BUP (90 mg/kg) in this study was not observed to produce seizures.

Statistical analysis: animal studies. Data sets were analyzed using Prism Software (Graph-Pad Software, San Diego, CA) to generate sigmoidal dose–response curves, ID50s (the estimated doses that result in 50% inhibition of food intake), and determine statistical significance. One-way ANOVA and Tukey's multiple comparison posttests were used to determine significant differences between treatment groups at specific time points. For drug coadministration studies, two-way ANOVA was used to analyze feeding data for drug additivity or synergy (for further description and review, see refs. 23,29). Specifically, the F statistic for an interaction effect in a two-way ANOVA is mathematically comparable to testing the null hypothesis (no interaction, which implies drug additivity); therefore, a significant interaction between drug-treated animals is indicative of a synergistic interaction (29). A P value of $P < 0.05$ was considered significant. Data are expressed as mean ± s.e.m.

Human proof-of-concept clinical trial

Subjects. This Phase 2 trial was a parallel, multicenter, randomized, blinded trial comparing placebo, NAL immediate release (IR) monotherapy, BUP sustained release (SR) monotherapy, and the BUP+NAL combination in subjects with uncomplicated obesity. The study was conducted from February 2004 through November 2004. The protocol was approved by each investigative site's institutional review board/ethics committee and each subject provided written informed consent before participation in any study-related procedures. The implementation of the study was consistent with Good Clinical Practice standard and the Declaration of Helsinki (World Medical Association, 2000). The primary treatment period was 16 weeks with an extension through week 24 for the BUP+NAL and BUP+P groups.

A total of 238 male and female subjects at least 18–60 years of age who had a BMI ≥30 and ≤40 kg/m^2, and who were free from clinically significant illness or disease as determined by medical history and physical examination participated in the study (**Figure 3**, **Table 1**). Subjects were healthy with no comorbid medical conditions (unstable and/or requiring concomitant therapy). Subjects had been nonsmokers for at least 6 months and were not permitted to use regular concomitant medications with the exception of hormone replacement, oral contraceptives, nonopioid analgesics, nonsteroidal anti-inflammatory agents, antiulcer medications (except cimetidine), vitamins, minerals, or intermittent allergy/cold medications. Subjects who were pregnant or lactating, had obesity of known endocrine origin, had prior surgical treatment for obesity, had a seizure disorder or predisposition to such, were smoking in the 6 months prior to the trial, had a history of an eating disorder, had a history of atherosclerotic vascular disease, abused drugs or alcohol in the 5 years before the trial, had a history of cancer (except basal cell skin cancer or cured cervical cancer), had a depression or anxiety score of ≥11 on the Hospital Anxiety and Depression Scale, had used BUP or NAL in the year prior to the trial, or used nonprescription medications, or supplements for weight loss in the month prior to the trial were excluded from the trial. Females of child-bearing potential agreed to use effective contraception throughout the study period.

Study design. The trial consisted of an initial screening phase where subjects provided a medical history and received a physical examination, electrocardiogram, waist circumference, fasting chemistry panel, complete blood count, urinalysis, thyroid stimulating hormone, and were administered the Hospital Anxiety and Depression Scale. Qualifying subjects were then blindly randomized into one of four treatment groups: BUP 300 mg SR and NAL 50 mg IR (BUP+NAL), BUP 300 mg SR and placebo (BUP+P), NAL 50 mg IR and placebo (NAL+P), and placebo and placebo (P+P) for 16 weeks. BUP or placebo BUP were titrated across the first 6 days, beginning with 150 mg BUP SR QD, and 150 mg BUP SR BID thereafter; NAL (50 mg QD) or placebo NAL were not titrated. Although a formal diet, exercise, and behavior modification intervention did not occur, subjects received instruction at baseline on a diet which was 500 kcal/day below maintenance based on the World Health Organization's algorithm for calculating resting metabolic rate. Adjusted body weight was used to calculate energy needs because the subjects' weights were 120% greater than ideal body weight. Study subjects were seen at baseline and study weeks 2, 4, 8, 12, and 16. During the 8-week extension (weeks 17–24), subjects, per protocol, in the BUP+NAL and BUP+P groups were continued on their blinded therapy. Given the expectation of minimal benefit and per the interest of site institutional review boards, those subjects in the NAL+P and double placebo groups were assessed only through week 16; thereafter, they could elect to crossover to open-label combination therapy (BUP+NAL).

Statistical analyses: clinical study. The sample size was calculated assuming the mean weight loss from baseline to week 16 would be ≤2% for the group receiving NAL monotherapy and no active treatment, ≤5% for the group receiving BUP monotherapy, and ≥7.5% for the group receiving BUP+NAL. A common standard deviation of 5% was assumed for all groups. A two-sample t-test was used to compare the groups according to the intention-to-treat (ITT) principle. It was estimated that 51 randomized subjects with at least one postbaseline weight measurement would be needed in each group to detect a ≥50% improvement in mean weight loss for the experimental combination treatment relative to its most active constituent, with a one-tailed significance level of 5%, and power of at least 0.80 for each test. The overall power for each of the three comparisons was 0.78.

Unless otherwise specified, all efficacy analyses were conducted on an ITT basis, which consisted of all subjects who were randomized with at least 1 postbaseline body weight measurement. Sensitivity analyses were conducted using a completer population. The completer population is defined as subjects that were taking both study medications at the specified time point (i.e., week 16 and week 24). All randomly assigned subjects who received at least 1 administration of study drug were included in the safety analyses.

The primary efficacy analysis was the percent change from baseline in total body weight, during the 16-week primary treatment period (week 16 ITT, last observation carried forward, LOCF). The efficacy associated with combination treatment was assessed relative to its individual constituents and to placebo. The Min test procedure was used to maintain the family-wise type I error rate to within the prespecified one-tailed α-level of 5% (30,31). The null hypothesis was simultaneously tested using three one-tailed comparisons. The results of all three tests had to be statistically significant at the 5% one-tailed α-level for the combination treatment to be considered superior to its individual constituents and placebo. Treatment group comparisons were based upon the least squares means (LS means) estimated by a linear fixed-effects analysis of covariance (ANCOVA) that included terms for treatment group, sex, and baseline BMI (hereafter referred to as the adjusted model). Additional secondary efficacy analyses on body weight used the ANCOVA model as described above for the primary analysis and included analyses such as percentage weight loss at week 24. Changes in obesity-associated cardiovascular risk factors were analyzed using an ANCOVA model that included terms for treatment group, sex, baseline BMI, and the baseline measurement for the appropriate cardiovascular risk factors. Rates of response (defined as ≥5% and ≥10% change in body weight from baseline) were analyzed with the Fisher's exact test, and the Min test procedure was performed to maintain the type I error rate as described previously. Safety assessments were summarized by treatment group.

RESULTS

Preclinical

Electrophysiology studies. BUP (10 μmol/l) reversibly increased the frequency of action potentials in POMC neurons (3 of 3 neurons tested; **Figure 1a**). The opioid antagonist NAL (1 μmol/l) also reversibly increased the frequency of action potentials in POMC neurons (10 of 13 neurons tested;

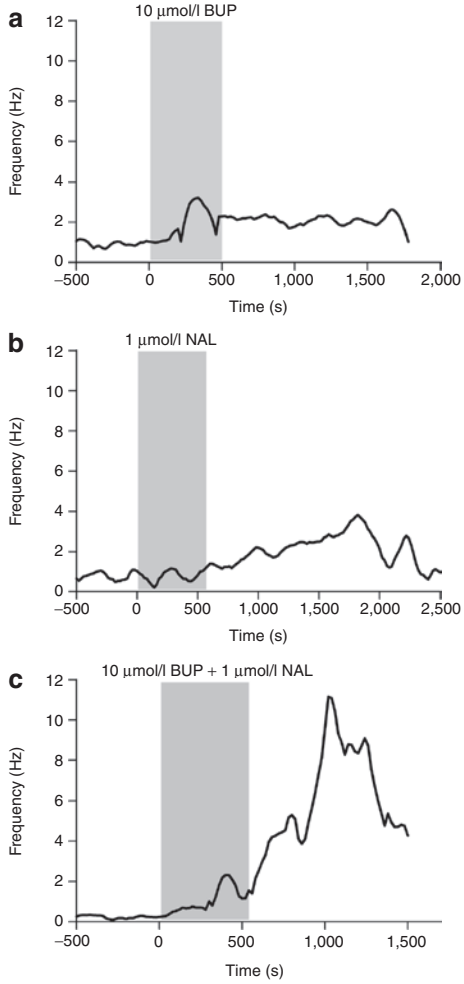


Figure 1 Bupropion (BUP) (10 µmol/l), naltrexone (NAL) (1 µmol/l), and BUP (10 µmol/l) + NAL (1 µmol/l) transiently increase the activity of POMC neurons. The frequency of action currents in identified POMC neurons was recorded by cell-attached patch electrophysiological recordings in brain slices taken from POMC–EGFP mice. Each tracing is a representative recording from one cell. Drug application, beginning at 0 s and lasting about 550 s, is denoted by a shaded region. (**a**) BUP (10 µmol/l) reversibly increased the frequency of action currents in a POMC neuron (representative of 3/3 cells tested). (**b**) NAL (1 µmol/l) reversibly increased the frequency of action currents in a POMC neuron (representative of 10/13 cells that responded to NAL). (**c**) BUP (10 µmol/l) + NAL (1 µmol/l) reversibly increased the frequency of action currents in a POMC neuron (representative of 3/3 cells tested).

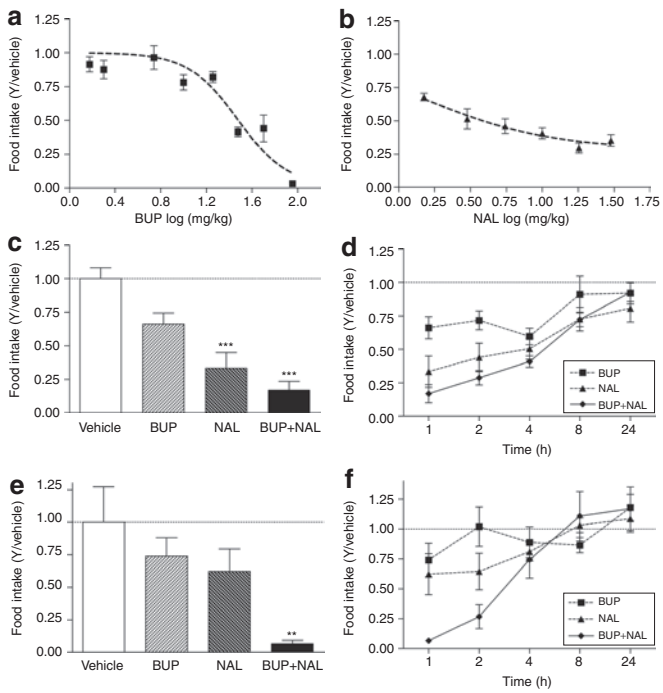


Figure 2 Coadministration of BUP and NAL acutely inhibits food intake in lean and obese mice. (**a,b**) Dose–response curves in lean mice on a normal diet. Data is normalized to vehicle. (**a**) Reduction in 1 h food intake by BUP (n = 5–10/group; R^2 = 0.64). (**b**) Reduction in food intake by NAL at 1 h postinjection. (n = 10/group; R^2 = 0.39). (**c–f**) 16 h fasted lean and DIO mice were treated with vehicle, BUP (20 mg/kg IP), NAL (1 mg/kg IP), or BUP (20 mg/kg) + NAL (1 mg/kg), and intake of high-fat chow was measured. Data is normalized to vehicle. (**c**) Effect of BUP, NAL, BUP+NAL, or vehicle on 1 h intake of high-fat chow in lean mice (n = 6–7/group). (**d**) Time course for effect of BUP, NAL, and BUP+NAL on high-fat chow intake in lean mice. (**e**) Effect of BUP, NAL, BUP+NAL, or vehicle on 1 h intake of high-fat chow in DIO mice (n = 5–7/group). (**f**) Time course for effect of BUP, NAL, and BUP+NAL on food intake in DIO mice. (Data is represented as mean ± s.e.m.; statistical analysis performed on raw data: $^{**}P < 0.01$, $^{***}P < 0.001$ for treatment compared to vehicle.)

Figure 1b). Combined application of BUP (10 µmol/l) + NAL (1 µmol/l) reversibly increased the firing frequency of POMC neurons (3 of 3 neurons; Figure 1c). In these experiments, each cell was its own control, and only stable cells were analyzed. This data illustrates a potential mechanism for an anorectic effect of BUP and NAL, and suggests that combination therapy with BUP+NAL may produce a greater combined effect on energy balance *in vivo*.

Animal studies. Fasted lean mice on a normal diet were treated with BUP (1.5, 3, 5.5, 10, 18, 30, 50, 90 mg/kg), NAL (1.5, 3, 5.5, 10, 18, or 30 mg/kg), or vehicle administered peripherally. BUP produced a dose-dependent reduction in feeding at 1 h with an ID50 of 35 mg/kg (Figure 2a; n = 5–10/group). The effect of BUP on feeding was transient; intake was similar to control by 4 h postinjection. All doses of NAL significantly reduced food intake at 1 h postinjection ($P < 0.05$); therefore, a dose–response curve was estimated by setting the upper constraint at 1 (null effect for NAL) (Figure 2b; n = 10/group). The estimated ID50 for NAL was 3.3 mg/kg. The ED50 (dose that caused half of the maximum effect of NAL or 35% reduction in food intake) was 1.7 mg/kg. For all doses of NAL, food intake was similar to baseline by 4 h postinjection.

Based on the above dose–response tests in lean mice, doses of BUP (20 mg/kg IP) and NAL (1 mg/kg IP) were chosen that would produce a moderate (≤50%) reduction in feeding at 1 h postinjection. Drugs were administered alone or in combination to 16 h food-deprived lean (n = 6–7/group) and obese (n = 5–7/group) mice on a high-fat diet. In lean control mice, NAL (1 mg/kg) significantly inhibited food intake at 1, 2, and 4 h postinjection ($P < 0.001$) and BUP+NAL significantly decreased food intake in lean mice at 1, 2, and 4 h postinjection ($P < 0.001$, Figure 2c,d). BUP (20 mg/kg) significantly

inhibited food intake only at 4 h postinjection ($P < 0.01$). Two-way ANOVA revealed a nonsignificant interaction and a significant main effect of BUP ($F(1,21)=8.22$; $P < 0.01$)) and NAL ($F(1,21)= 3.88$; $P < 0.01$)) at 1 h (and 2 h), indicating that NAL and BUP alone significantly inhibited food intake, and the combined effect of these agents was at least fully additive.

Neither NAL nor BUP alone had a significant effect on food intake in DIO mice at any time point (**Figure 2e,f**); however, the combination (BUP+NAL) significantly decreased food intake at 1 and 2 h postinjection as analyzed by one-way ANOVA ($P < 0.01$). Two-way ANOVA revealed a significant main effect of BUP ($F(1,19) = 6.60$; $P < 0.05$)) and NAL ($F(1,19) = 10.93$; $P < 0.01$)) at 1 h postinjection, but no significant interaction, demonstrating that that the combination is at least fully additive in obese mice. However, two-way ANOVA has been hypothesized to be an underpowered analysis of synergy for small sample sizes; a larger sample size may result in a significant interaction (23,29). In the absence of a significant effect of either BUP+P or NAL+P on short-term feeding (as assessed by one-way ANOVA), the statistically significant and substantial reduction in food intake caused by the BUP+NAL combination is indicative of drug synergy. Daily caloric intake was similar between animals on normal and high-fat diet.

Human proof-of-concept trial

Study subjects, baseline characteristics, and disposition. A total of 238 subjects were randomized (**Figure 3**). The majority of subjects were female (89%) and white (66%). The average age was 42 years. At baseline, the average body weight was 95 kg with an average BMI of 35 kg/m^2. Baseline characteristics were well-balanced among the four treatment groups (**Table 1**). A total 217 subjects (91%) received at least 1 administration of study treatment (**Figure 3**), and 207 had at least 1 postbaseline body weight measurement (**Table 2**).

Weight changes. **Figure 4** illustrates percent weight loss by ITT-LOCF and **Figure 5** shows percent weight loss for study completers at both 16 and 24 weeks. Of the four groups, the greatest weight loss was in the BUP+NAL group. For both ITT and completers analysis, the percent change in total body weight at week 16 was significantly different for the BUP+NAL group compared with the NAL+P ($P < 0.01$) and P+P ($P < 0.001$) treatment groups. Although a diverging weight loss trajectory was evident in the BUP+NAL vs. BUP+P groups at week 16, the difference was not statistically significant (**Table 2**). At 24 weeks, the percent weight change from baseline in the BUP+NAL group was further increased and numerically greater than BUP+P but was nonsignificant in the ITT population (**Table 2**; **Figure 4**). However, in the completer population (**Figure 5**), BUP+NAL demonstrated an unadjusted significant difference relative to the BUP+P ($P = 0.045$).

Responder analyses. At 16 weeks, combination therapy was associated with a statistically significant greater number of subjects achieving ≥5% weight loss compared to P+P and NAL+P in the ITT analysis and compared to P+P in the completers analysis (**Table 2**). For ≥10% weight loss in the ITT responder analysis, there was a statistically significant difference between the rate of responders in the BUP+NAL and NAL+P groups.

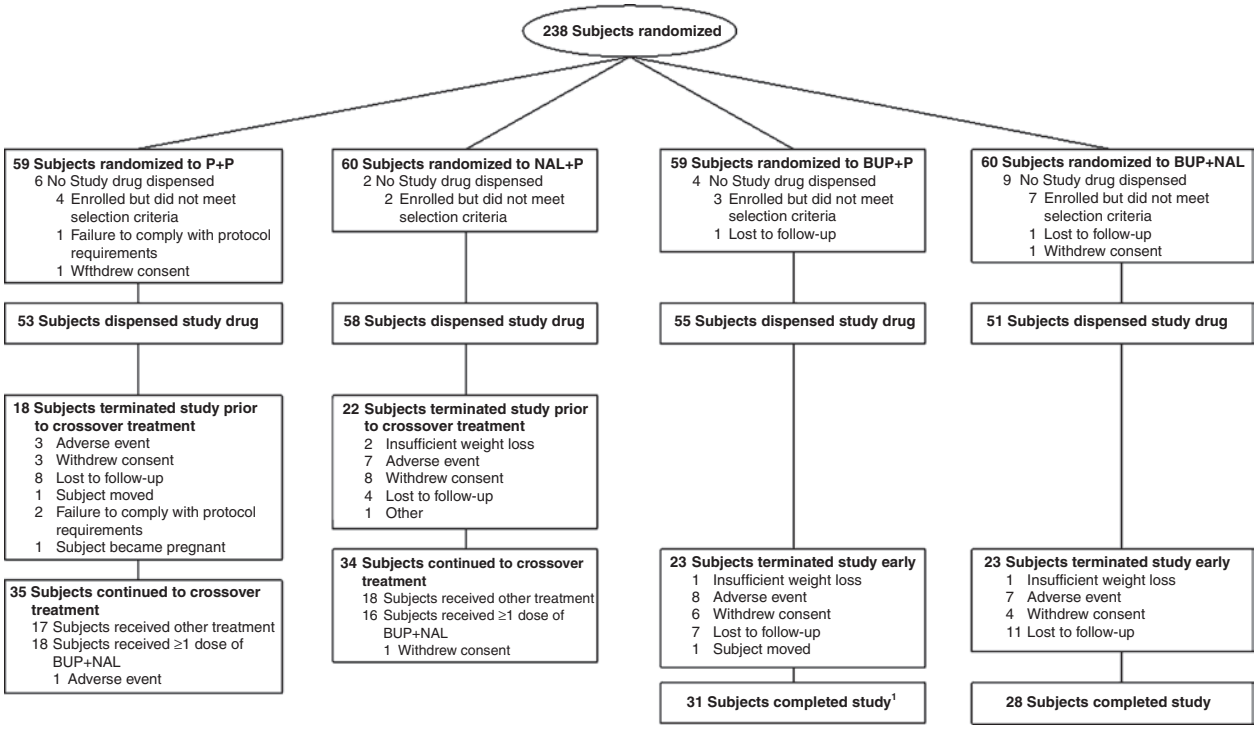


Figure 3 Subject flow diagram. [1]One additional, off-drug subject was allowed to continue the study.

Table 1 Subject demographics and other baseline characteristics

Characteristics	P+P (*N* = 59)	NAL+P (*N* = 60)	BUP+P (*N* = 59)	BUP+NAL (*N* = 60)
Age (years): Mean (s.d.)	41 (9.9)	40 (10.2)	44 (9.8)	43 (9.7)
Gender (female): *n* (%)	52 (88%)	54 (90%)	53 (90%)	53 (88%)
Race: *n* (%)				
White	37 (63%)	41 (68%)	43 (73%)	37 (61.7%)
Black or African American	20 (34%)	19 (32%)	13 (22%)	22 (36.7%)
Other	2 (3%)	0	3 (5%)	1 (1.7%)
Efficacy Parameters: Mean (s.d.)				
Weight (kg)	95 (10.3)	96 (12.6)	94 (9.8)	96 (14.0)
BMI (kg/m^2)	35 (2.9)	35 (2.8)	35 (3.2)	35 (3.2)
Waist circumference (cm)	102 (10.5)	102 (13.0)	101 (10.8)	102 (12.7)
HADS-A subscale score	2.7 (2.5)	3.4 (2.3)	3.7 (2.3)	3.4 (2.7)
HADS-D subscale score	1.8 (2.3)	1.9 (2.2)	2.3 (2.3)	2.3 (2.2)

HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale-Depression subscale; *N*, Number of randomized subjects.

These comparisons were nonsignificant among completers. At 24 weeks, there was a nonsignificant trend for more BUP+NAL subjects to attain a >5% or >10% weight loss than their BUP+P counterparts (both the ITT and completer analyses); however, these differences did not reach significance (Table 2).

Secondary efficacy measures. Change for obesity-associated cardiovascular risk factors (waist circumference, vital signs, and blood glucose) between baseline and week 16 were similar in magnitude across all treatment groups except for waist circumference (Table 2). Waist circumference numerically declined in all drug treatment groups and was increased in the P+P group; at week 16, waist circumference was significantly different only between BUP+NAL and P+P ($P < 0.0001$). For the other risk factors, the LS mean changes from baseline were nonsignificant between the treatment groups.

Withdrawals and adverse events. There were three serious adverse events (none were attributed to study drug by the investigator); two in the BUP+P group (viral meningitis and inguinal hernia), and one in the NAL+P group (deep vein thrombosis). There were no study deaths. A total of 56 participants withdrew during the first 16 weeks from the following groups: BUP+NAL, 14 (23%); BUP+P, 8 (14%); NAL+P, 18 (30%); and P+P, 16 (27%). Another 24 subjects withdrew from the study during weeks 17–24 BUP+NAL, 9 (15%); BUP+P, 15 (25%). The most common reasons for early termination at week 16 were subject loss to follow-up (9% of all subjects) and adverse events (8% of all subjects). Table 3 summarizes adverse events exceeding 5% for any group during weeks 1–16 and the number of adverse events resulting in discontinuation of drug. These were consistent with the existing package literature on the individual agents perhaps best illustrated by nausea which is a characteristic adverse event associated with NAL IR. Nausea was the most common adverse event and the adverse event that most commonly led to discontinuation of active treatment. The discontinuation rate due to nausea was highest in the NAL+P ($n = 8$; 14%) group at week 16. Hospital Anxiety and Depression Scale subscale scores did not demonstrate any treatment-related adverse effects on anxiety or depression scores across any treatment group.

DISCUSSION

In this report, we present a series of studies designed to test the hypothesis that combination treatment with BUP+NAL is associated with greater weight loss than the individual agents or an inactive control. Moreover, we postulated that the addition of NAL would alter the anticipated early plateau in weight loss associated with BUP. Electrophysiological recordings in POMC neurons *in vitro*, *in vivo* feeding studies in mice, and a clinical trial in obese subjects all consistently demonstrated that the BUP+NAL combination was associated with a greater treatment effect than either monotherapy or placebo. Specifically, preclinical studies demonstrated a positive effect of combination BUP+NAL treatment on POMC cell firing rates and an additive, possibly synergistic, inhibitory effect of BUP and NAL on food intake in lean and obese mice. In obese human subjects, combination treatment produced greater weight loss than either monotherapy alone. Notably, subjects treated with BUP+NAL appeared to exhibit continued weight loss through week 24 in contrast to the plateau observed in subjects receiving BUP+P. Collectively, these results suggest that addition of an opioid antagonist (NAL) to a POMC activator (BUP) potentiates weight loss in obese humans.

In the arcuate nucleus, posttranslational processing of the POMC prohormone results in two primary peptide products, α-melanocyte–stimulating hormone (α-MSH), and β-endorphin (6), and α-MSH mediates the anorexic effects typically associated with stimulation of POMC cells (6,32). In contrast, μ-opioid agonists like β-endorphin inhibit POMC cells (7,12,13) and increase food intake in rodents (33,34). Blockade of μ-opioid receptors can produce anorexic effects in animals and humans (35–37) and the opioid antagonist, NAL, increases

Table 2 Body weight and cardiovascular risk factors

	P+P	NAL+P	BUP+P	BUP+NAL
Week 16: Body weight				
ITT population[a]	*N* = 51	*N* = 53	*N* = 53	*N* = 50
Unadjusted % change: LSMean (s.e.)[b]	–0.6 (0.5)***	–1.7 (0.5)**	–3.2 (0.5)	–3.7 (0.5)
Adjusted % change: LSMean (s.e.)[b]	–1.0 (0.6)***	–2.0 (0.6)**	–3.6 (0.6)	–4.0 (0.6)
≥5% weight loss: *n* (%)	6 (12%)*	8 (15%)*	14 (26%)	16 (32%)
≥10% weight loss: *n* (%)	2 (4%)	0 (0%)*	3 (6%)	5 (10%)
Completers[c]	*N* = 38	*N* = 34	*N* = 40	*N* = 34
Unadjusted % change: LSMean (s.e.)[b]	–0.8 (0.7) ***	–2.1 (0.7)**	–3.7 (0.7)	–4.6 (0.7)
Adjusted % change: LSMean (s.e.)[b]	–1.0 (0.8)***	–2.3 (0.8)**	–3.9 (0.8)	–4.8 (0.8)
≥5% weight loss: *n* (%)	6 (16%)*	7 (21%)	12 (30%)	14 (41%)
≥10% weight loss: *n* (%)	2 (5%)	0 (0%)	2 (5%)	4 (12%)
Week 16: Cardiovascular Risk Factors: LSMean (s.e.)[d]				
Diastolic BP (mm Hg)	1.3 (1.2)	–1.5 (1.2)	0.7 (1.2)	1.0 (1.2)
Systolic BP (mm Hg)	1.3 (1.7)	–0.4(1.7)	3.2(1.7)	1.3(1.7)
Fasting glucose (mg/dl)	1.1 (1.0)	0.9 (1.0)	1.3 (1.0)	1.7 (1.0)
Pulse rate (beats/min)	–1.6 (1.1)	–3.2 (1.1)	–1.6 (1.1)	–0.2 (1.1)
Waist circumference (cm)	1.1 (1.0)***	–1.4 (1.0)	–3.7 (1.0)	–3.0 (1.0)
Week 24: Body weight				
ITT population[a]	N/A	N/A	*N* = 53	*N* = 50
Unadjusted % change: LSMean (s.e.)[b]	N/A	N/A	–3.1 (0.7)	–4.4 (0.8)
Adjusted % change: LSMean (s.e.)[b]	N/A	N/A	–4.0 (1.0)	–5.2 (1.0)
≥5% weight loss: *n* (%)	N/A	N/A	15 (28%)	18 (36%)
≥10% weight loss: *n* (%)	N/A	N/A	5 (9%)	6 (12%)
Completers[e]	N/A	N/A	*N* = 31	*N* = 28
Unadjusted % change: LSMean (s.e.)[b]	N/A	N/A	–4.0 (1.0)*	–6.5 (1.1)
Adjusted % change: LSMean (s.e.)[b]	N/A	N/A	–4.5 (1.3)	–6.8 (1.2)
≥5% weight loss: *n* (%)	N/A	N/A	11 (35%)	15 (54%)
≥10% weight loss: *n* (%)	N/A	N/A	3 (10%)	5 (18%)
Week 24: Cardiovascular Risk Factors: LSMean (s.e.)[d]				
Diastolic BP (mm Hg)	N/A	N/A	–1.3 (1.4)	–0.8 (1.4)
Systolic BP (mm Hg)	N/A	N/A	–1.4 (1.8)	–0.7 (1.8)
Fasting glucose (mg/dl)	N/A	N/A	2.9 (1.1)	2.6 (1.1)
Pulse rate (beats/min)	N/A	N/A	–0.8 (1.2)	–1.1 (1.2)
Waist circumference (cm)	N/A	N/A	–4.5 (1.0)	–3.7 (1.0)

[a] Intention-to-treat analysis of full analysis set; data accounted for by LOCF. [b]Unadjusted model: least squares means (LSMeans) estimated from an ANOVA model. Adjusted model: LSMeans estimated from ANCOVA model which included terms for treatment group, sex and baseline BMI. [c]Completers defined as subjects who received at least 16 weeks of treatment. [d]Change from baseline: LSMeans estimated from an ANCOVA model which include terms for treatment group, sex, baseline BMI, and baseline measurement for the appropriate CV risk factor. [e]Completers defined as subjects who received at least 24 weeks of treatment.
Pairwise comparisons vs. BUP+NAL group: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

activation of melanocortin neurons in the arcuate nucleus (38). Collectively, this data supports a β-endorphin-mediated mechanism for autoinhibition of arcuate POMC neurons.

In our electrophysiological study, blockade of opioid receptors with NAL increased activity of POMC cells, probably by removing tonic β-endorphin-mediated inhibition of POMC neurons. Consistent with the anorexic effect of BUP in rodents and humans (11,24), the dopamine and norepinephrine reuptake inhibitor also stimulated POMC firing above basal rates. Furthermore, the effect of combined application of BUP+NAL on POMC firing rate was also evident and in agreement with an autoinhibitory effect of β-endorphin. The exact mechanism for BUP-mediated stimulation of POMC cells requires further investigation but appears to be due to combined dopaminergic and noradrenergic stimulation (22–24). Although preliminary, electrophysiological studies provided

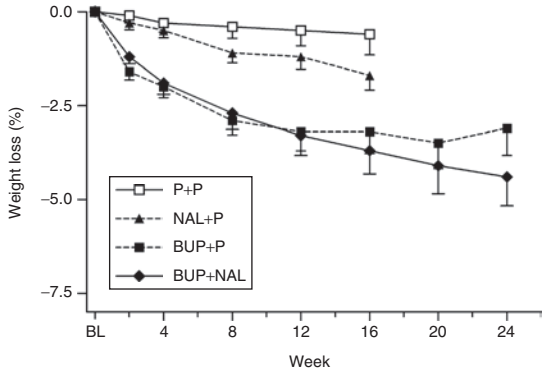


Figure 4 Intent-to-treat population. Mean (–s.e.m.) percent change in total body weight from baseline (BL) through study weeks 16 (P+P and NAL+P) and 24 (BUP+P and BUP+NAL).

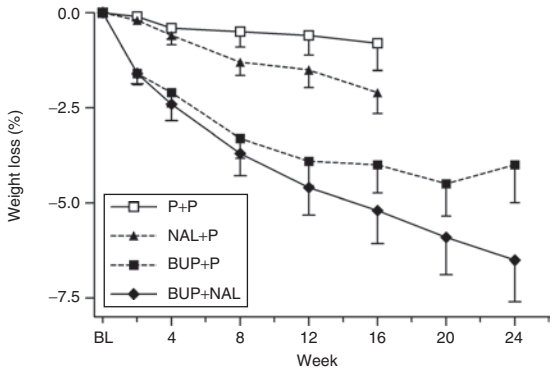


Figure 5 Completer population. Mean (–s.e.m.) percent change in total body weight from baseline (BL) through study weeks 16 (P+P and NAL+P) and 24 (BUP+P and BUP+NAL).

Table 3 Adverse events

	P+P (*N* = 53) *n* (%)	P+NAL (*N* = 58) *n* (%)	BUP+P (*N* = 55) *n* (%)	BUP+NAL (*N* = 51) *n* (%)
Subjects with > 1TEAE	19 (35.8%)	32 (55.2%)	30 (54.5%)	29 (56.9%)
Nausea	1 (1.9%)	20 (34.5%)	5 (9.1%)	16 (31.4%)
Headache	7 (13.2%)	2 (3.4%)	10 (18.2%)	8 (15.7%)
Dizziness	2 (3.8%)	6 (10.3%)	5 (9.1%)	7 (13.7%)
Fatigue	4 (7.5%)	5 (8.6%)	5 (9.1%)	5 (9.8%)
Vomiting NOS	1 (1.9%)	8 (13.8%)	3 (5.5%)	3 (5.9%)
Diarrhea NOS	0 (0.0%)	2 (3.4%)	4 (7.3%)	5 (9.8%)
Insomnia	0 (0.0%)	1 (1.7%)	3 (5.5%)	7 (13.7%)
Back pain	3 (5.7%)	0 (0.0%)	2 (3.6%)	2 (3.9%)
Nasopharyngitis	2 (3.8%)	2 (3.4%)	3 (5.5%)	0 (0.0%)
Gastroenteritis NOS	1 (1.9%)	2 (3.4%)	3 (5.5%)	0 (0.0%)
Dry mouth	0 (0.0%)	1 (1.7%)	3 (5.5%)	1 (2.0%)
Irritability	0 (0.0%)	0 (0.0%)	3 (5.5%)	0 (0.0%)
Paraesthesia	3 (5.7%)	2 (3.4%)	0 (0.0%)	0 (0.0%)
Abdominal pain (upper)	0 (0.0%)	1 (1.7%)	0 (0.0%)	3 (5.9%)
Subjects who discontinued due to adverse event[a]	5 (9.4%)	14 (24.1%)	9 (16.4%)	9 (17.6%)

Incidence >5% in at least 1 treatment group with onset during weeks 1–16.
N, number of randomized subjects; *n*, number of subjects with event; TEAE, treatment-emergent adverse event.
[a]Discontinuation from 1 or both study drugs during weeks 1–16 due to adverse events starting during weeks 1–16.

a proof-of-concept for drug synergy and a potential cellular mechanism for the anorexic effects of BUP and NAL.

Increased POMC signaling in rodents has been associated with decreased appetite, increased metabolism, and weight loss, whereas decreased POMC signaling is associated with hyperphagia and energy conservation (6). The current feeding studies in mice produced results consistent with increased POMC activity in the presence of BUP+NAL. The acute anorexic effects of BUP and NAL in lean and obese mice also replicate previously published data (23,39–42); however, the evidence for the combined inhibitory effect of BUP+NAL on food intake in lean and obese mice is novel. Body weight also appeared to be a relevant treatment variable, with BUP+NAL demonstrating a greater and possibly synergistic reduction in food intake in obese mice compared to lean mice. One interpretation of this finding is that basal activity of POMC neurons is lower in obese mice, possibly due to obesity-induced leptin resistance in POMC neurons (8,9). The addition of BUP could be expected to increase POMC firing and enhance the capacity of NAL to prevent autoinhibition of POMC neurons, resulting in sustained activity. This idea is consistent with the marked reduction in feeding caused by the combination in **Figure 2**. It is important to note that neither NAL nor BUP monotherapies had a statistically significant effect on food intake, while the NAL+BUP combination caused a large and statistically significant reduction in food intake in obese mice, demonstrating drug synergy. Therefore, our preclinical *in vivo* results provided evidence that treatment of obese human subjects with BUP+NAL should be

associated with greater weight loss than has already been documented with BUP or NAL monotherapy (11).

In the clinical proof-of-concept trial, BUP+NAL was indeed associated with greater weight loss than either the individual components or placebo. Although the 16-week weight loss with BUP+NAL was not significantly greater than with BUP+P, a significant difference was observed between these two groups in the unadjusted completer population by week 24; further divergence between the two treatments could be expected with a longer study. The lack of significance in a BUP+NAL vs. BUP+P comparison at the primary endpoint (week 16) may also have been attributable to the limited sample size in this proof-of-concept investigation. A previous trial comparing BUP monotherapy at doses of 300 mg/day and 400 mg/day vs. placebo in obese subjects demonstrated that the weight-loss trajectory for subjects receiving BUP 300 mg/day (the dose used in this study) appeared to plateau by 20 weeks of treatment (11). In our study, subjects receiving BUP+P also exhibited this early weight-loss plateau in contrast to subjects receiving the combination treatment (**Figures 4** and **5**). The progressive weight loss observed in the BUP+NAL group through week 24 suggests that continued administration of BUP+NAL beyond 24 weeks in a future study may result in additional weight loss and further differentiation from BUP alone.

The combination of BUP+NAL was generally well tolerated. There were no drug-related serious adverse events, and no meaningful effects of treatment on laboratory analytes, electrocardiograms, or vital signs. The most commonly reported adverse event was nausea; the frequency was numerically highest in the NAL IR group followed by the combination therapy group. Nausea was typically early in onset, mild, and transient. It was infrequently associated with emesis. Other common adverse events included headache, dizziness, and insomnia. Nausea is common in subjects taking NAL IR (43) and may be due to both a local prokinetic effect in the stomach and at the level of the chemotrigger zone in the medulla. These are likely a function of the T_{max} (gut) and C_{max} (central) of the IR NAL formulation. Contributions are likely from both the parent compound and NAL's primary metabolite, 6β-naltrexol (44). A previous positron emission tomography study suggests that 50 mg of NAL provides a very high degree of saturation and sustained (>72 h) occupancy of central opiate receptors (45). Therefore, we would speculate that lower doses of NAL and/or a reformulation into a SR might reduce the frequency of nausea.

Some important limitations of these studies should be considered in the interpretation of our findings. Although electrophysiological recording demonstrated that both NAL and BUP and the combination stimulate POMC neurons, not enough recordings were performed to provide statistical analysis of a firing rate that would allow for quantitative comparison of the firing rate between individual drugs and the combination. This type of analysis requires holding the cell for sufficient time to allow application of all treatments, a complication exacerbated by the fact that BUP is a reuptake inhibitor and must be applied for a longer duration. Therefore, we chose to test whether the electrophysiological data supported the idea of synergy and test for synergy in the preclinical and clinical studies. Preclinical *in vivo* studies provided adequate data for statistical analysis of drug additivity or synergy. The lack of a significant main effect for BUP+NAL in lean mice did not support effects beyond drug additivity; however, combination BUP+NAL treatment appeared to be more potent in DIO mice, suggesting that the BUP+NAL combination may be synergistic and, therefore, more effective in an obese model. A larger sample size may have provided sufficient statistical strength for a synergistic interaction. Interpretation of animal studies is also limited by the variable effect of NAL on food intake, as the inhibitory effect of NAL appeared to be reduced in obese mice on a high-fat diet. Although the nontraditional (nonsigmoidal) dose–response curve for NAL in lean animals and the lack of a subsequent dose–response study in obese mice on a high-fat diet limits a direct comparison for efficacy, the small effect of NAL on feeding in obese mice is similar to the negligible effect of NAL on body weight in humans.

The most important limitation to the clinical study was sample size; as with many proof-of-concept studies the *a priori* estimation of the effect size is challenging. We suspect this limited sample size and the failure of approximately one-third of subjects to reach the primary endpoint may have prevented significant differences between combination therapy and BUP monotherapy at week 16. Consistent with the pattern of separation of their respective weight loss curves, more robust differences were observed by week 24. Most of the subjects were white females; *post hoc* descriptive statistics on percent weight change from baseline by gender (data not shown) indicated numerically greater weight loss in male subjects in the BUP+NAL group. The small number of males enrolled does not allow immediate conclusions to be drawn from this observation. These findings suggest an appropriate future trial might be of longer duration and with a greater number of male subjects in order to more fully explore the BUP+NAL hypothesis.

In this series of studies, we provide evidence for a novel and synergistic benefit of NAL in enhancing the effects of BUP on weight loss. These studies demonstrate a stepwise approach to mechanistic hypothesis testing in a true translational sense, beginning with an *in vitro* electrophysiologic model, progressing to studies in animal physiology, and concluding with a randomized clinical trial aimed at translating the preclinical findings into human populations. Future clinical studies are encouraged to both replicate and extend these findings. These studies also support the use of an opioid antagonist as a potential adjunct to BUP in the treatment of obesity. Whether this will be the case with other POMC activators requires testing. The benefit reported here highlights some of the complex neuronal circuitry mediating not only acute appetite and body weight regulation, but also compensatory mechanisms that may mitigate longer-term, meaningful clinical results with diet and/or monotherapeutic drug approaches.

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DISCLOSURE

F.L.G., M.J.W., M.G., K.M.G., E.D., G.D.T, E.W., and M.A.C. are employees of, or consultants to, Orexigen Therapeutics, Inc., and may hold stock in the company. The work described in this article was, in part, supported by Orexigen Therapeutics Inc. OHSU and M.A.C. have a significant financial interest in Orexigen Therapeutics Inc., a company that may have a commercial interest in the results of this research and technology. This potential conflict has been reviewed and managed by the OHSU Conflict of Interest in Research Committee and the Integrity Program Oversight Council.

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REFERENCES

1. Ogden CL, Carroll MD, Curtin LR *et al*. Prevalence of overweight and obesity in the United States, 1999–2004. *JAMA* 2006;295:1549–1555.
2. Flegal KM, Graubard BI, Williamson DF, Gail MH. Cause-specific excess deaths associated with underweight, overweight, and obesity. *JAMA* 2007;298:2028–2037.
3. Yan LL, Daviglus ML, Liu K *et al* Midlife body mass index and hospitalization and mortality in older age. *JAMA* 2006;295:190–198.
4. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA* 2004;291:1238–1245.
5. Stein CJ, Colditz GA. The epidemic of obesity. *J Clin Endocrinol Metab* 2004;89:2522–2525.
6. Cone RD. Anatomy and regulation of the central melanocortin system. *Nat Neurosci* 2005;8:571–578.
7. Cowley MA, Smart JL, Rubinstein M *et al*. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* 2001;411:480–484.
8. Munzberg H, Flier JS, Bjorbaek C. Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. *Endocrinology* 2004;145:4880–4889.
9. Enriori PJ, Evans AE, Sinnayah P *et al*. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. *Cell Metab* 2007;5:181–194.
10. Aronne LJ, Thornton-Jones ZD. New targets for obesity pharmacotherapy. *Clin Pharmacol Ther* 2007;81:748–752.
11. Anderson JW, Greenway FL, Fujioka K *et al*. Bupropion SR enhances weight loss: a 48-week double-blind, placebo-controlled trial. *Obes Res* 2002;10:633–641.
12. Ibrahim N, Bosch MA, Smart JL *et al*. Hypothalamic proopiomelanocortin neurons are glucose responsive and express K(ATP) channels. *Endocrinology* 2003;144:1331–1340.
13. Kelly MJ, Loose MD, Ronnekleiv OK. Opioids hyperpolarize β-endorphin neurons via mu-receptor activation of a potassium conductance. *Neuroendocrinology* 1990;52:268–275.
14. Riddle M. Combining sulfonylureas and other oral agents. *Am J Med* 2000;108(Suppl 6a):15S–22S.
15. Gradman AH, Acevedo C. Evolving strategies for the use of combination therapy in hypertension. *Curr Hypertens Rep* 2002;4:343–349.
16. Weintraub M, Hasday JD, Mushlin AI, Lockwood DH. A double-blind clinical trial in weight control. Use of fenfluramine and phentermine alone and in combination. *Arch Intern Med* 1984;144:1143–1148.
17. Connolly HM, Crary JL, McGoon MD *et al*. Valvular heart disease associated with fenfluramine-phentermine. *N Engl J Med* 1997;337:581–588.
18. Wadden TA, Berkowitz RI, Womble LG *et al*. Effects of sibutramine plus orlistat in obese women following 1 year of treatment by sibutramine alone: a placebo-controlled trial. *Obes Res* 2000;8:431–437.
19. Ascher JA, Cole JO, Colin JN *et al*. Bupropion: a review of its mechanism of antidepressant activity. *J Clin Psychiatry* 1995;56:395–401.
20. Foley KF, DeSanty KP, Kast RE. Bupropion: pharmacology and therapeutic applications. *Expert Rev Neurother* 2006;6:1249–1265.
21. Hasegawa H, Meeusen R, Sarre S *et al*. Acute dopamine/norepinephrine reuptake inhibition increases brain and core temperature in rats. *J Appl Physiol* 2005;99:1397–1401.
22. Pelletier G. Regulation of proopiomelanocortin gene expression in rat brain and pituitary as studied by in situ hybridization. *Ann N Y Acad Sci* 1993;680:246–259.
23. Billes SK, Cowley MA. Inhibition of dopamine and norepinephrine reuptake produces additive effects on energy balance in lean and obese mice. *Neuropsychopharmacology* 2007;32:822–834.
24. Billes SK, Cowley MA. Catecholamine reuptake inhibition causes weight loss by increasing locomotor activity and thermogenesis. *Neuropsychopharmacology* 2008; 33:1287–1297.
25. Bouza C, Angeles M, Munoz A, Amate JM. Efficacy and safety of naltrexone and acamprosate in the treatment of alcohol dependence: a systematic review. *Addiction* 2004;99:811–828.
26. Atkinson RL, Berke LK, Drake CR *et al*. Effects of long-term therapy with naltrexone on body weight in obesity. *Clin Pharmacol Ther* 1985;38: 419–422.
27. Malcolm R, O'Neil PM, Sexauer JD *et al*. A controlled trial of naltrexone in obese humans. *Int J Obes* 1985;9:347–353.
28. Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord* 2000;24:639–646.
29. Slinker BK. The statistics of synergism. *J Mol Cell Cardiol* 1998;30:723–731.
30. D'Agostino RB, Massaro J, Kwan H, Cabral H. Strategies for dealing with multiple treatment comparisons in confirmatory clinical trials. *Drug Inf J* 1993;27:625–641.
31. Laska EM, Meisner MJ. Testing whether an identified treatment is best. *Biometrics* 1989;45:1139–1151.
32. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* 1997;385:165–168.
33. Grandison L, Guidotti A. Stimulation of food intake by muscimol and beta endorphin. *Neuropharmacology* 1977;16:533–536.
34. Kalra SP, Horvath TL. Neuroendocrine interactions between galanin, opioids, and neuropeptide Y in the control of reproduction and appetite. *Ann N Y Acad Sci* 1998;863:236–240.
35. Holtzman SG. Suppression of appetitive behavior in the rat by naloxone: lack of effect of prior morphine dependence. *Life Sci* 1979;24:219–226.
36. Yeomans MR, Gray RW. Opioid peptides and the control of human ingestive behaviour. *Neurosci Biobehav Rev* 2002;26:713–728.
37. Zhang J, Frassetto A, Huang RR *et al*. The μ-opioid receptor subtype is required for the anorectic effect of an opioid receptor antagonist. *Eur J Pharmacol* 2006;545:147–152.
38. Olszewski PK, Wirth MM, Grace MK, Levine AS, Giraudo SQ. Evidence of interactions between melanocortin and opioid systems in regulation of feeding. *Neuroreport* 2001;12:1727–1730.
39. Zarrindast MR, Hosseini-Nia T. Anorectic and behavioural effects of bupropion. *Gen Pharmacol* 1988;19:201–204.
40. Recant L, Voyles NR, Luciano M, Pert CB. Naltrexone reduces weight gain, alters "β-endorphin", and reduces insulin output from pancreatic islets of genetically obese mice. *Peptides* 1980;1:309–313.
41. Bodnar RJ. Endogenous opioids and feeding behavior: a 30-year historical perspective. *Peptides* 2004;25:697–725.
42. Cooper SJ. Naloxone: effects on food and water consumption in the non-deprived and deprived rat. *Psychopharmacology* (Berl) 1980;71:1–6.
43. Croop RS, Faulkner EB, Labriola DF. The safety profile of naltrexone in the treatment of alcoholism. Results from a multicenter usage study. The Naltrexone Usage Study Group. *Arch Gen Psychiatry* 1997;54:1130–1135.
44. King AC, Volpicelli JR, Gunduz M, O'Brien CP, Kreek MJ. Naltrexone biotransformation and incidence of subjective side effects: a preliminary study. *Alcohol Clin Exp Res* 1997;21:906–909.
45. Lee MC, Wagner HN Jr, Tanada S *et al*. Duration of occupancy of opiate receptors by naltrexone. *J Nucl Med* 1988;29:1207–1211.