



Trip Report for

**“Keystone Symposium on Neuronal Mechanisms Controlling Food
Intake, Glucose Metabolism and Body Weight,”
Fairmont Banff Springs, Banff, Alberta, Canada, February 19–24, 2008**

Matthew D. Surman, Ph.D.

April 21, 2008

AMRI Memorandum

TO: Ahmed, Feryan; Barnes, Keith; Carr, Grant; Chase, Matthew; Earley, William G.; Geiss, William B.; Gregg, Brian T.; Guaciaro, Michael; Guzzo, Peter R.; Herr, R. Jason; Khmelnsky, Yuri; Kitchen, Douglas B.; Liu, Shuang; Luche, Michele M.; Manning, David D.; Michels, Peter; Quinn, John F.; Reilly, John E.; Shenoy, Rajesh A.; Wolf, Mark A.; Yang, Zhicai; Yeung, Raymond; Zhichkin, Pavel

FROM: Matthew D. Surman, Ph.D.

DATE: February 19–24, 2008

RE: “Keystone Symposium on Neuronal Mechanisms Controlling Food Intake, Glucose Metabolism and Body Weight,” Fairmont Banff Springs, Banff, Alberta, Canada

Abstract: The focus of this symposium was on the cellular and molecular mechanisms of neural circuit integration in the perception of satiety, regulation of body weight and control of insulin sensitivity. The symposium shared several joint plenary and poster sessions with the concurrent “Molecular Control of Adipogenesis and Obesity” symposium. The following text summarizes some of the highlights of the symposia.

“The Emerging Biology of Taste Perception,”

Charles Zuker (University of California, San Diego), San Diego, CA

Prof. Zuker kicked off the symposium with a keynote address covering taste perception. Mammals perceive five basic tastes: sweet, sour, bitter, umami (savory), and salt. Each taste provides valuable information about materials that are ingested. Sweet offers evaluation of caloric content; sour warns of spoiled food; bitter warns of toxic substances; umami gives information on amino acid content; and salt assists with electrolyte balance. The basic anatomical unit for taste detection is the taste receptor cell (TRC). TRCs are organized into taste buds in specific areas of the tongue. However, the taste buds contain cells that respond to all tastes. Therefore, contrary to popular belief, there is no tongue “map” for different tastes.

The attractive taste modalities (sweet and umami) are mediated by three GPCRs (G-protein coupled receptors), T1R1, T1R2, and T1R3. TRCs co-expressing T1R2 and T1R3 (combined to form the heteromeric T1R2+3 receptor) are responsible for sensing all classes of sweet tastants, including natural sugars (sucrose, fructose, etc.), artificial sweeteners (saccharin, aspartame, etc.), D-amino acids, and intensely sweet proteins (monellin, thaumatin, etc.). T1R-sequence variation between species imparts significant differences in the ability to taste certain artificial sweeteners and intensely sweet proteins.

For example, mice cannot taste aspartame or monellin. However, introduction of the human T1R2 receptor into T1R2 knockout mice confers these animals with the ability to detect “human” sweet tastants such as aspartame and monellin. As with sweet taste receptors, umami receptors are made up of heterodimers of two T1R GPCRs. In the case of umami, T1R1 and T1R3 combine to form T1R1+3. In most mammals, T1R1+3 receptors detect a broad range of L-amino acids. However, human T1R1+3 receptors selectively respond to only two amino acids, monosodium glutamate (MSG) and aspartate.

Bitter tastes are detected by a large family of approximately 30 GPCRs, the T2Rs. This large and highly divergent set of receptors allows for the recognition of a wide range of toxic compounds, all of which invoke a common “bitter” taste sensation. The different T2Rs respond to specific bitter tastants, as demonstrated by the observation that animals lacking a particular T2R do not have the ability to taste the associated bitter compound. Additionally, animals can be engineered to respond to particular unnatural bitter tastes. For example, mice do not naturally respond to “human” bitter tastants phenylthiocarbamide or salicin, since they lack the corresponding T2Rs. However, introduction of the human T2R receptors (T2R38 and T2R16, respectively) into mice results in vigorously aversive reactions to these chemicals. The TRCs expressing T2Rs are distinct from those expressing sweet and umami receptors. Additionally, most, if not all, T2Rs are expressed on the same TRCs.

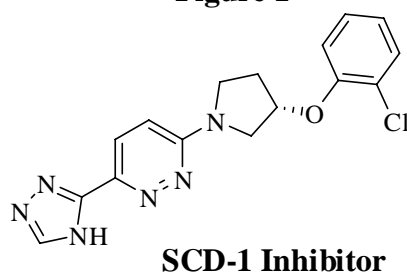
Unlike the GPCRs responsible for sweet, umami, and bitter taste detection, the sour receptor is believed to be PKD2L1, a member of the TRP channel family. The TRCs expressing PKD2L1 are distinct from those expressing the T1Rs or the T2Rs. In fact, TRCs mediating sweet, umami, bitter, and sour tastes are highly segregated in the periphery and no overlap exists between cells expressing different taste receptors. This observation supports a “labeled-line” model for taste encoding in the periphery. In this model, receptor cells respond to single taste modalities and are innervated by individually tuned nerve fibers. Opposing models involve an “across-fiber pattern,” in which either a) individual TRCs respond to multiple taste modalities (therefore the nerve fibers send signals for multiple tastes) or b) the TRCs respond to single tastes, but common nerve fibers carry information for more than one taste from multiple TRCs. Additional evidence for the “labeled-line” model comes from experiments in which a single taste modality can be deleted (sweet, bitter, or sour) by eliminating entire populations of specific TRCs (expressing T1R2, T2R, or PKD2L1, respectively) while keeping all other taste responses. Additionally, mice engineered with a bitter receptor in sweet TRCs show a strong attraction to the associated bitter compound. This indicates that the perception of taste is mediated by dedicated cells hard wired to stereotypical behavior, rather than by a property of the receptors or by the tastant, itself.

“Pharmacological Inhibition of SCD-1 as a Potential Therapeutic Target for Obesity”

Claire Steppan (Pfizer), Groton, CT

Stearoyl-CoA desaturase (SCD) is a microsomal enzyme that catalyzes the rate limiting step in the biosynthesis of mono-unsaturated fatty acids from saturated fatty acids. SCD introduces a cis-double bond in the Δ -9 position of saturated 16 and 18-carbon fatty acid CoAs, thereby converting palmitoyl- and stearoyl-CoAs into palmitoleoyl- and oleoyl-CoAs, respectively. Four isoforms of SCD have been identified in mice (SCD-1, SCD-2, SCD-3 and SCD-4). In humans, two isoforms have been identified (SCD-1 and SCD-5). SCD-1 knockout mice show increased energy expenditure, increased insulin sensitivity, reduced body adiposity and resistance to diet induced obesity and hepatic steatosis. Based upon the favorable attributes of the SCD-1 knockout mouse phenotype, Pfizer pursued inhibitors of SCD-1 for the treatment of obesity. A high throughput mass spectroscopy assay was used to identify hit compounds. The assay employed rat liver microsomes as the enzyme source.

Figure 1



Results from chronic DIO rat feeding study

Dose	10 mg/kg qd	10 mg/kg bid	3 mg/kg bid
Decrease in body fat	33%	49%	38%
Reduction in cumulative food intake	7.1%	10.1%	8.7%
Reduction in plasma saturation index	22%	25%	17%

Data was presented for a potent, selective SCD-1 inhibitor (Figure 1). The compound showed in vitro potency of approximately 100 nM at hSCD1 and approximately 30 nM at rSCD. Statistically significant weight loss was observed in a 21 day DIO rat study at 3 and 10 mg/kg twice daily doses, but not at a 10 mg/kg once daily dose. The weight loss was associated with a decrease in body fat. Interestingly, the reduction in body weight could be attributed to a decrease in food intake. Reductions in the plasma desaturation index (ratio of monounsaturated to saturated fatty acids) provided evidence of in vivo SCD inhibition. However, no significant changes were observed in respiratory quotient, plasma triglycerides, liver triglycerides, insulin levels or glucose levels. Additionally, degeneration of the ocular harderian and meibomian glands were seen. Based upon these data, the conclusion was made that in adult DIO rats, SCD-1 inhibitors replicate only a portion of the SCD-1 knockout mouse phenotype.

“In Vitro and In Vivo Pharmacological Characteristics of Lorcaserin, a Novel and Selective 5-HT_{2C} Agonist”

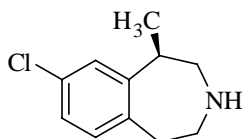
Andrew Grottick (Arena Pharmaceuticals), San Diego, CA

The centrally located serotonin 5-HT_{2C} receptor has been identified as an important mediator of satiety. Mice lacking the 5-HT_{2C} receptor are hyperphagic and mildly obese. A number of non-selective 5-HT_{2C} agonists (such as fenfluramine, part of the infamous “fen-phen” weight loss drug combination) lead to weight loss in rodents and humans due to reduced food intake. The effect of these non-selective agents are reduced or absent in 5-HT_{2C} receptor knockout mice.

The 5-HT_{2C} receptor shares considerable sequence homology with the closely related 5-HT_{2A} and 5-HT_{2B} receptors. Activation of central 5-HT_{2A} receptors has been linked to altered perception and hallucinations. Activation of 5-HT_{2B} receptors in the cardiovascular system has been implicated in the cardiac valvulopathy associated with non-selective serotonergic agents, such as fenfluramine. Selectively activating the 5-HT_{2C} receptor, while leaving the 5-HT_{2A} and 5-HT_{2B} receptors unaffected has been a major challenge in pursuing 5-HT_{2C} receptor agonists for the treatment of obesity.

Arena Pharmaceuticals is developing Lorcaserin, a selective and high affinity 5-HT_{2C} agonist (Figure 2). Lorcaserin acts as a full agonist at the human 5-HT_{2C} receptor in a functional inositol phosphate accumulation assay and shows 20-fold selectivity over 5-HT_{2A} and 100-fold selectivity over 5-HT_{2B}. Importantly, Lorcaserin did not induce behaviors indicative of functional 5-HT_{2A} agonist activity. Lorcaserin also showed selectivity for 5-HT_{2C} over a panel of more than 70 GPCRs and ion channels, including 5-HT_{1A}, 5-HT_{1B}, 5-HT₃, 5-HT_{4C}, 5-HT_{5A}, 5-HT₆, and 5-HT₇.

Figure 2



Lorcaserin

5-HT_{2C} EC₅₀ = 7.9 nM

5-HT_{2A} EC₅₀ = 158 nM

5-HT_{2B} EC₅₀ = 794 nM

Results from acute food intake study in rat

Dose	2.4 mg/kg (12.5 µmol/kg)	4.9 mg/kg (25 µmol/kg)	9.8 mg/kg (50 µmol/kg)	19.6 mg/kg (100 µmol/kg)
% Inhibition at 2 h post dose	34 ± 3	58 ± 4	77 ± 5	82 ± 6

Results from chronic 28 day HFD rat feeding study

Dose	18 mg/kg qd	9 mg/kg bid	18 mg/kg bid	36 mg/kg bid
Decrease in body weight gain	3.3%	4.6%	6.3%	8.5%

Lorcaserin showed dose-dependent inhibition of food intake when given acutely to rats (Figure 2). This effect could be reversed by pre-administration of the selective 5-HT_{2C} receptor antagonist SB242086, but not by the 5-HT_{2A} antagonist M100,907. Therefore, the reduction in food intake was shown to be associated with selective mediation of the 5-HT_{2C} receptor.

When given chronically to rats maintained on a high fat diet (HFD), Lorcaserin caused dose-dependent reductions in food intake and body weight gain (Figure 2). While the reductions in food intake only lasted for the first 7 days, the reductions in body weight gain remained throughout the course of the 28 day study. The 36 mg/kg twice daily dose resulted in an overall 8.5% reduction in weight gain compared with vehicle. After administration of Lorcaserin was terminated, the body weight gradually returned to control levels.

Lorcaserin showed statistically significant, progressive and dose dependent weight loss over a 12 week period in phase II clinical trials. No apparent effects on heart valves or pulmonary artery pressure were observed. Lorcaserin is currently in phase III clinical trials.

Reference: Brian M. Smith, et al. *J. Med. Chem.* **2008**, *51*, 305-313.

“Comparison of Lorcaserin and Sibutramine in a High-Fat Diet Mouse Model”

Juergen Prestle (Boehringer-Ingelheim Pharma GmbH & Co. KG),
Biberach a.d.R., Germany

Lorcaserin is a 5-HT_{2C} receptor agonist currently in Phase III clinical development for the treatment of obesity (*vide supra*). In rat models of obesity, Lorcaserin shows similar efficacy to that of Sibutramine. The current study focused on profiling Lorcaserin in mice.

The *in vitro* potency of Lorcaserin was determined by agonist-induced Ca²⁺-mobilization in CHO cell lines expressing human 5-HT_{2C}, 5-HT_{2A} and 5-HT_{2B} (Table 1). Lorcaserin was found to have 23-fold selectivity for 5-HT_{2C} over 5-HT_{2A} and 13-fold selectivity over 5-HT_{2B}.

Table 1
Lorcaserin *in vitro* potency

Agonist activity, EC ₅₀ (nM)	h5-HT _{2C}	h5-HT _{2A}	h5-HT _{2B}
Lorcaserin	35 nM (E _{max} * = 96%)	819 nM (E _{max} * = 18%)	473 nM (E _{max} * = 79%)
5-HT	3 nM	54 nM	3 nM

*100% E_{max} = stimulation with 5-HT (1 μM)

A modified Irwin test battery for CNS adverse events was carried out in NMRI mice at single oral doses of Lorcaserin of 10, 30, and 100 mg/kg. The 10 mg/kg dose did not cause any adverse behavioral effects. The 30 and 100 mg/kg doses caused transient and dose-dependent ataxia and decreased motor activity. Lorcaserin (10, 30 and 100 mg/kg, once daily) was administered for 14 days to male NMRI mice maintained on a high fat diet (HFD) and compared with Sibutramine (5 and 20 mg/kg, once daily) (Table 2). The Lorcaserin-treated groups showed minor, non-significant decreases in body weight. Sedation-like effects were observed in the 30 and 100 mg/kg dose groups during the first day. The effects were transient in nature and did not continue on subsequent days. NMR measurement of whole body composition demonstrated normalization of body fat content to chow-fed controls with the Sibutramine treated groups. The Lorcaserin groups did not show any effect on body composition compared to the HFD controls.

Table 2
Results from sub-chronic 14 day HFD NMRI mouse feeding study

Dose (po, qd)	Lorcaserin 10 mg/kg	Lorcaserin 30 mg/kg	Lorcaserin 100 mg/kg	Sibutramine 5 mg/kg	Sibutramine 20 mg/kg
Decrease in body weight vs. HFD control	1.3% (n.s.)†	1.2% (n.s.)†	3.1% (n.s.)†	7.3% (p<0.01)	11.5% (p<0.001)

†n.s. = not significant

In summary, Sibutramine and Lorcaserin show similar clinical efficacy in humans. However, unlike Sibutramine, Lorcaserin showed little effect on body weight and body

composition in HFD mice. Therefore, the HFD mouse model may not be an effective predictor for the 5-HT_{2C} receptor mechanism.

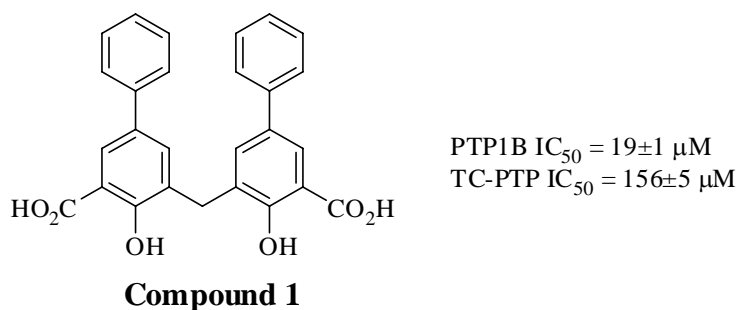
“Disalicylic Acid Derivatives as PTP1B Inhibitors Exhibit Anti-hyperglycemic Effects and Also Inhibit IKK-β”

Bharat Raj Bhattarai, (Inha University), Incheon, Republic of Korea.

Protein tyrosine phosphatase 1B (PTP1B) is a negative regulator of the insulin signaling pathway. PTP1B knockout mice have significantly lower plasma insulin levels than wild type mice and undergo increased glucose disposal in oral glucose or insulin tolerance tests. The knockout mice have low body fat and are resistant to weight gain when maintained on a high fat diet. Therefore, PTP1B inhibitors are being pursued for the treatment of type 2 diabetes and obesity.

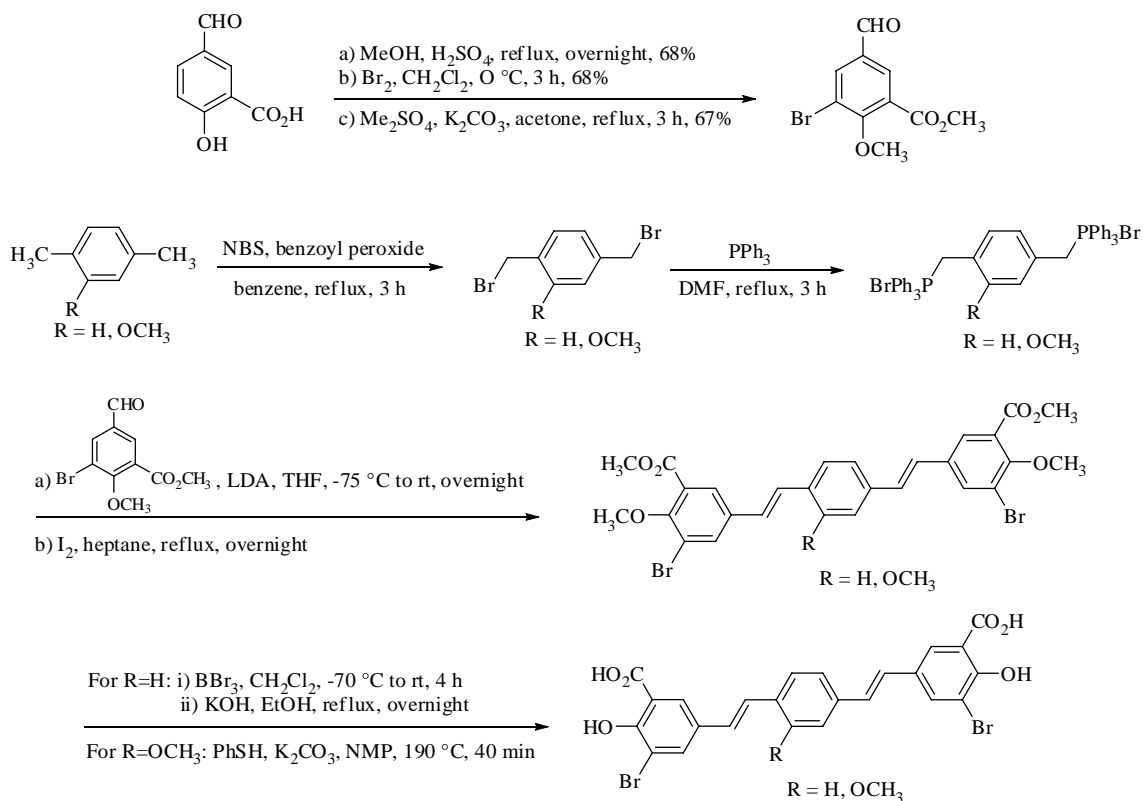
In the early to mid 1900s, high doses of salicylic acid or aspirin were used to reduce the symptoms of type 2 diabetes. More recently, polymeric substances with multiple salicylic acid moieties have been found as inhibitors of PTP1B. For example, methylenedisalicylic acid derivative **1** (Figure 3) was found to produce significant reductions in weight gain in high fat diet (HFD) mice.¹ When given mixed with food at approximately 400 mg/kg/day, compound **1** reduced weight gain by 13% compared with HFD controls. The reduced weight gain was associated with reduced fat pad mass. No significant differences were measured in food intake between the compound-treated and control groups, indicating the reduced weight gain was associated with increased energy expenditure.

Figure 3



The current study focused on disalicylic acids with stilbene or distyrylbenzene backbones. The synthesis of several of the distyrylbenzene analogues is shown in Figure 4.

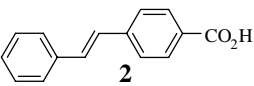
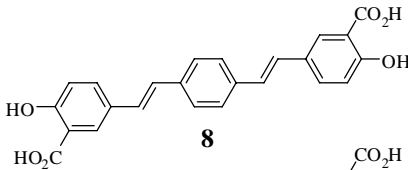
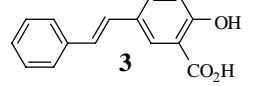
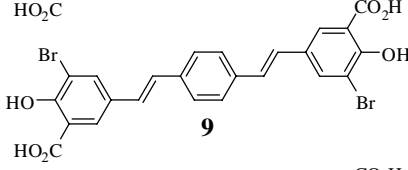
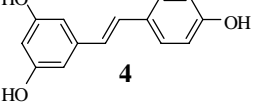
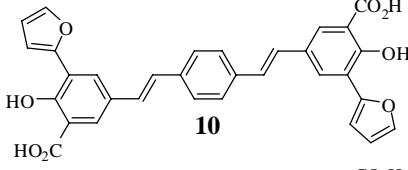
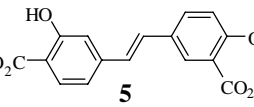
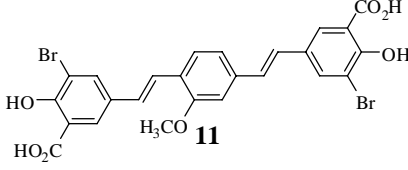
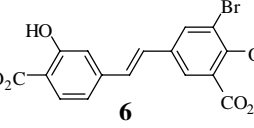
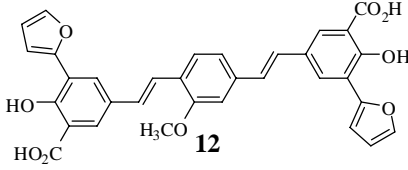
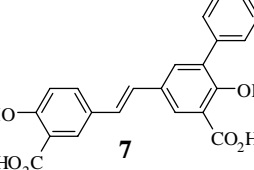
Figure 4



The salicylic acid functionality appeared to be critical for PTP1B activity (Figure 5, compare compound **3** with compounds **2** and **4**). The presence of a second salicylic acid on the stilbene core did not change the potency of compound **5** compared with compound **3**. The addition of a bromide in compound **6** had a modest detrimental effect compared to compound **5**. Enhancements in potency were observed with the distyrylbenzene backbone (compare compound **8** with compounds **3** and **5**). Furanyl substitution provided a 10-fold improvement in PTP1B potency (compare compounds **8** and **10**).

Figure 5

In vitro PTP1B and IKK- β data

Structure	PTP1B IC ₅₀ (μ M)	Structure	PTP1B IC ₅₀ (μ M)	IKK- β IC ₅₀ (μ M)
	708 \pm 8		5 \pm 0.4	1.3
	34 \pm 4		3 \pm 0.2	0.57
	>1000		0.5 \pm 0.1	0.069
	32 \pm 5		6 \pm 0.4	0.54
	64 \pm 11		4 \pm 0.4	0.080
	125 \pm 18			

Compound **9** was studied in a 4-week high fat diet-induced diabetic mouse model. The compound was supplied as a 0.2% w/w mixture with food. Chronic administration of compound **9** did not significantly affect food intake or body weight. However, significant improvements were observed in fasting glucose levels and glucose tolerance. No significant changes in behavior or overt toxicity in liver and other organs were seen.

Compound **9** and many of the other distyrylbenzene compounds were found to inhibit IKK- β with submicromolar IC₅₀ values (Figure 5). Since insulin resistance involves the fat-induced activation of IKK- β , the relevant target for the in vivo effects of compound **9** remains unclear.

¹Hyeongjin Cho, et al. *Bioorg. Med. Chem.* **2007**, *15*, 6535-6548.

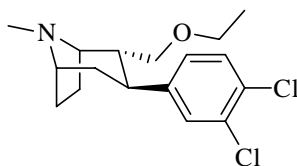
“The Novel Triple Monoamine Uptake Inhibitor Tesofensine Potently Reduces Body-Weight in Diet-Induced Obese (DIO) Rats: Comparison to Effects of Sibutramine and Rimonabant”

Jens D. Mikkelsen (NeuroSearch A/S) Denmark

Tesofensine is a serotonin-noradrenalin-dopamine reuptake inhibitor that exhibits relatively strong effects on all three monoaminergic systems. Originally under clinical development for the treatment of Alzheimer’s disease and Parkinson’s disease, Tesofensine is currently under development for the treatment of obesity.

The current study focused on comparing the effects of Tesofensine to the effects of weight loss drugs Sibutramine and Rimonabant on Levin-Rheoscience DIO rats. During the course of the 28 day study, Tesofensine demonstrated sustained body weight loss and decreased cumulative food intake. At doses of 1 mg/kg/day and 2.5 mg/kg/day, Tesofensine showed comparable weight loss to Sibutramine (7.5 mg/kg/day) and greater weight loss than Rimonabant (10 mg/kg/day) (Figure 6). No weight loss was observed with Tesofensine in lean rats. The DIO rats treated with Tesofensine showed significantly reduced total fat mass (27% for 1 mg/kg/day and 37% for 2.5 mg/kg/day) compared with vehicle-treated animals. The Tesofensine-treated animals also showed reductions in plasma triglycerides and cholesterol, as well as improved glycemic control.

Figure 6



Tesofensine

Results from 28 day DIO rat feeding study

Dose (po, qd)	Tesofensine 1 mg/kg	Tesofensine 2.5 mg/kg	Sibutramine 7.5 mg/kg	Rimonabant 10 mg/kg
Decrease in body weight vs. vehicle control	12%	16%	14%	5%

In a 203-patient, 24-week Phase IIb clinical study (“TIPO-1”), Tesofensine, in combination with a reduced calorie diet and exercise program, showed significant reductions in body weight of 6.5%, 11.2%, and 12.6% at doses of 0.25 mg, 0.5 mg, and 1.0 mg, respectively. The placebo group showed 2.0% weight loss. In a 32-patient, 14-day study (“TIPO-2”), Tesofensine (accelerated dosing up to 1 mg exposure) showed an average weight loss of 2.2 kg compared with 0.4 kg for the placebo group. NeuroSearch is currently preparing to progress Tesofensine into Phase III clinical trials.