New molecular targets for the Pharmacotherapy of Obesity

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Introduction

Current weight loss strategies are characterized by lack of success in the long term for the majority of patients. On the other hand, the public-health implications of the obesity pandemic state, makes the need of effective anti-obesity therapeutic intervention imperative. Pharmacotherapy of obesity however is a story full of pain filled with unsafe and abuse practices but also failures and withdraws of antiobesity drugs mainly due to poor safety and efficacy profile. The most recent withdrawals of fenfluramine and dexfenfluramine (related with valvulopathy), rimonabant (related with epilepsy and suicides) and sibutramine (related with adverse cardiac adverse) just proved the rule applied to anti-obesity drug records. The recent gains however in our understanding of energy homeostasis and the complex central and peripheral mechanisms that underlie the balance between energy intake and expenditure provide more effective molecular targets for obesity. The new drugs need to fulfill the strict safety and efficacy standards established by FDA for the development of anti-obesity pharmacotherapeutics requiring trials of ≥1 year duration that include >4,500 subjects (3,000 subjects randomized to active doses of the product and no fewer than 1,500 subjects randomized to placebo) (1). To consider a weight-loss pill for approval, the FDA looks for at least a 5% reduction in weight over a year. The FDA's ruling led the last few months to rejection three weight loss drugs, raising questions about whether any new drugs in the class can be made safe enough to win approval. It is now more than a decade that FDA has not approved a new diet pill.

Targeted Pharmacotherapy of Obesity

Novel insights provided by the new biology suggest the presence of a complex homeostatic system in which information about the energy reserve status and the meal quality and content is relayed from the periphery (gastrointestinal tract, pancreas and adipose tissue) via specific orexigenic and anorexigenic peptides and hormones to the central nervous system (CNS) (figure 1), which in turn drives or not feeding according to energy requirements (figure 2). This

knowledge, allows novel anti-obesity drugs to be designed targeting specific molecules implicated in energy status modulation. There are currently several pharmacological agents in development that result in weight loss, either by a reducing food intake, or by increasing energy expenditure.

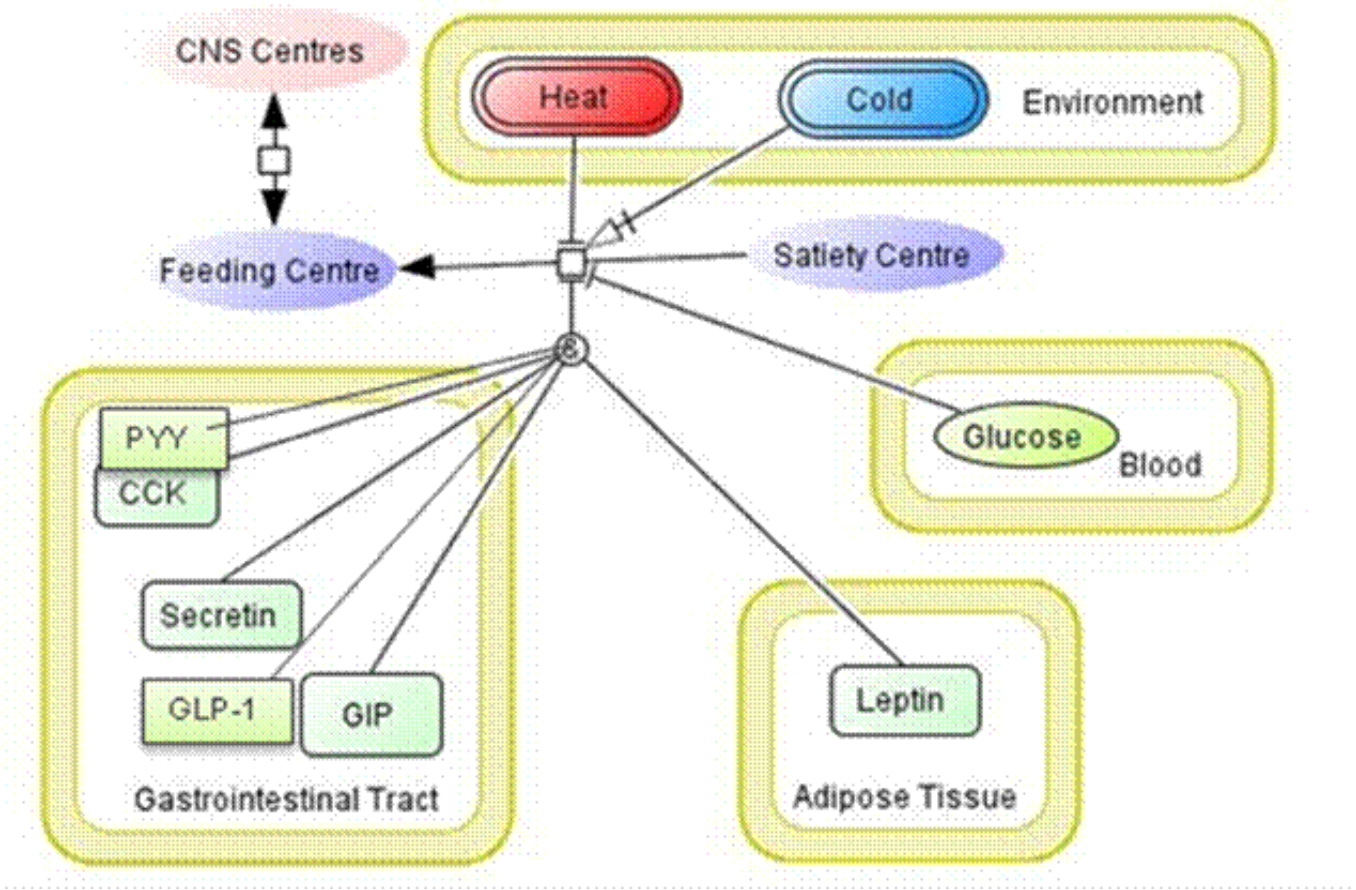

Figure 1. Appetite control : messengers from the gastrointestinal tract (e.g. CCK, secretin and GIP), from the pancreas (insulin) and from adipose tissue (leptin) converge upon the hypothalamus and provide a symbolic representation of the feeding status of the organism. These messages are then translated into either a food-seeking (orexic) or a fasting (anorexic) behaviour.
PYY: Peptide YY
CCK: Cholecystokinin
GIP: glucose-dependent insulinotropic peptide or gastric inhibitory polypeptide
GLP-1: Glucagon-like peptide-1

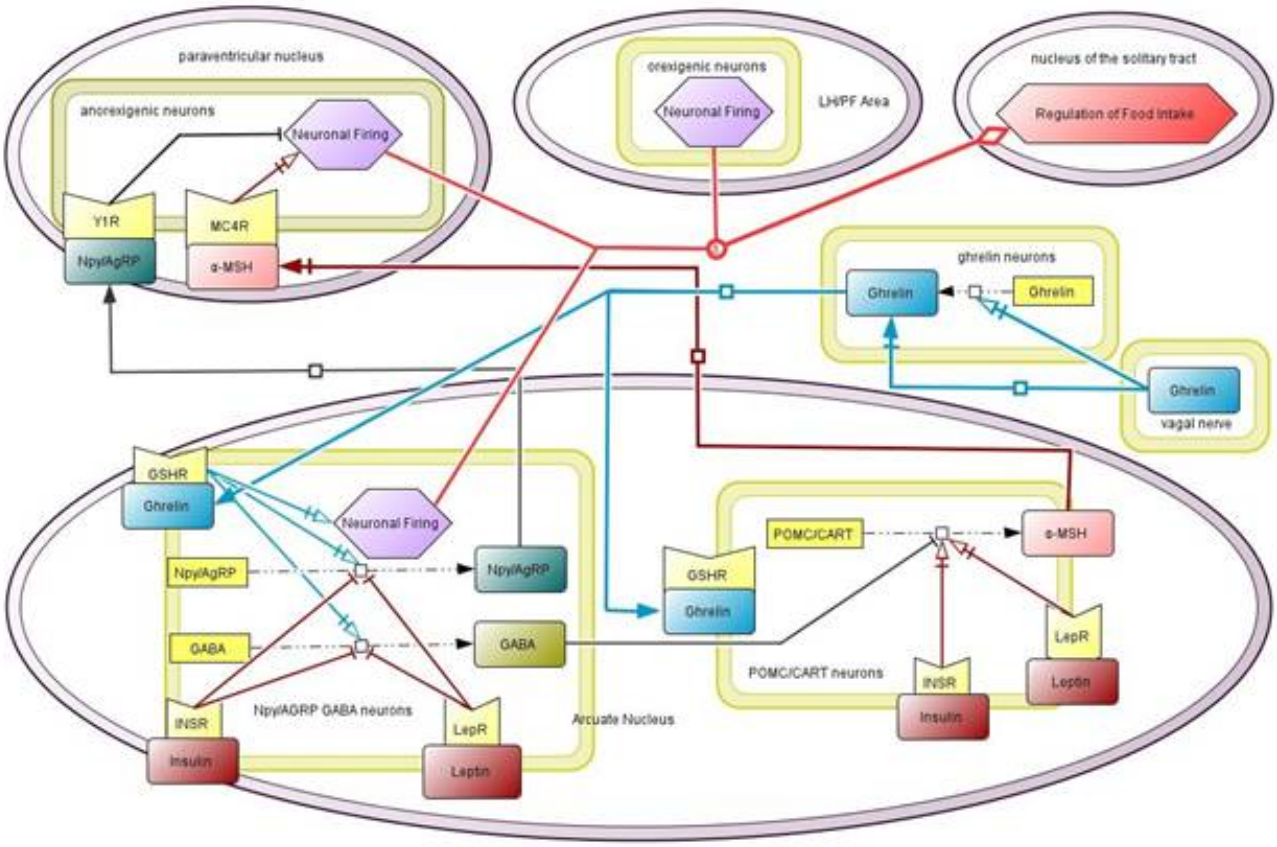

Figure 2. Regulation of food intake at the level of hypothalamus (arcuate nucleus). The orexigenic message of ghrelin enters the brain via the vagus nerve and causes local release of ghrelin from ghrelin-containing neurons which in turn binds to its receptor on Npy/AgRP/GABA neurons, giving rise to an increased production of Npy and AgRP as well as an increased firing rate of these neurons. The consequences are twofold: firstly, inhibition of firing of POMC/CART neurons (through GABA) and secondly stimulation of firing of the orexigenic neurons in the LHA/PFA region (through Npy and AgRP). A predominant orexigenic signal ensues.
Insulin and leptin directly diffuse into the arcuate nucleus and they bind to their receptors on both Npy/AgRP/GABA and POMC/CART neurons. However, they have opposing effects on these neurons. Leptin and insulin promote expression of α-MSH (anorexigenic) but suppress expression of Npy and AgRP (orexigenic). As a consequence a predominant anorexigenic signal ensues.
Messenger abbreviations : Npy, neuropeptide Y, AgRP, agouti-related protein, GABA, gamma-amino butyric acid; α-MSH, alpha-melanocyte stimulating hormone=melanocortin
Receptor abbreviations : GSHR, growth hormone-secretagogue receptor=ghrelin receptor; LepR, leptin receptor; INSR, insulin receptor; Y1R, Npy receptor, MC4R, melanocortin-4 (α-MSH) receptor.

Centrally-Acting Anti-Obesity Drugs / Modulators of Monoamine

Neurotransmission

Leptin, leptin analogues and leptin sensitizers

Leptin is a protein primarily secreted from the adipose tissue. It directly stimulates the anorexigenic proopiomelanocortin (POMC) neurons and inhibits the orexigenic neuropeptide Y (NPY) neurons in the arcuate nucleus (ARC) of hypothalamus promoting satiety and weight loss (2). Its circulating levels increase with increasing adiposity (3) and reduce following body weight reduction; the latter might be implicated in total and resting energy expenditure reduction seen after weight loss. Interestingly, even a diet-induced fall in leptin, not necessarily following weight loss, may dampen the anorexigenic effect of hindbrain satiation signals (4,5). Studies with congenitally leptin-deficient super-obese subjects revealed that administration of physiological doses of **leptin** decreases food intake and body weight (6,7). Based on these findings, restoration of leptin concentrations to preweight-loss concentrations, via administration of **metreleptin** (leptin analogue), has been shown to mitigate weight-loss counter-regulation (8-10). Putting together leptin may be an effective antiobesity therapy. Obese individuals however, despite having increased circulating leptin levels, they are leptin-resistant. Whether administration of leptin could overcome leptin resistance and exert anti-obesity effect was tested in a placebo-controlled study with 47 obese men and women given varying doses of **recombinant human leptin** (0.03 mg/kg and 0.30 mg/kg, respectively) for 24 weeks and advised to eat 500 kcal less than requirement each day. A weakly dose-dependent decrease in body weight was shown, ranging from -1.3 kg in the placebo group to -1.4 kg in the 0.03 mg/kg rh leptin-treated group and to -7.1 kg in the 0.30 mg/kg rh leptin-treated group (11) (194). Similar were the reports from animal studies testing the effect of the leptin resistance promoter protein tyrosine phosphatase-1B (**PTP1B**) **blockage** (12,13) or the chemical chaperones' that resolve ER stress and increase leptin sensitivity, including 4-phenyl butyric acid (**PBA**) and tauroursodeoxycholic acid (**TUDCA**) (14); they resulted in decreases in food intake, and reductions in body weight. Whether theses anti-obesity effects can be sustained or not is still not clear though. Therefore, it seems currently more probable a role for leptin as adjuvant therapy to a primary weight loss agent to maintain weight loss by reversing the total and resting energy expenditure reduction seen after weight reduction.

Recently, leptin-sensitizing effects were attributed to pramlintide, a synthetic analogue of the pancreatic peptide hormone amylin (see below). The anti-obesity properties of the combined treatment with pramlintide and the human hormone leptin analog metreleptin (**pramlintide/metreleptin**) were therefore tested showing significant weight reduction of 12.7 ± 0.9% (11.5 ± 0.9 kg) without plateau in obese patients during a 20-week trial period (15). Amylin Pharmaceuticals, Inc. subsequently announced positive results from a 28-week, proof-of-concept study with pramlintide and metreleptin combination treatment in overweight or obese subjects reporting that at the end of the study, the combination treatment reduced body weight on average 12.7%, significantly more than treatment with pramlintide alone (8.4%) which is translated to about 10 pounds more of weight loss with the combined treatment. Remarkably, subjects receiving pramlintide/metreleptin continued to lose weight through the end of the study compared to those treated with pramlintide alone, whose weight loss had stabilized towards the end of the study. The magnitude of weight loss was found to be dosage- and baseline BMI-

dependent while patients with a starting BMI less than 35 kg/m2, experienced the best weight loss efficacy of the combined treatment (16). A year later, Amylin Pharmaceuticals, Inc. announced the results of the 52-week blinded, placebo-controlled Phase 2 extension study of pramlintide/metreleptin suggesting sustained and robust weight loss by the combined treatment; again, the most robust efficacy was seen in patients with a body mass index (BMI) less than 35 kg/m2 (15). However, although the pramlintide/metreleptin combination seemed to be the next promising anti-obesity drug to be marketed, Amylin Pharmaceuticals, Inc. and Takeda Pharmaceutical Company Limited however, discontinued its development following commercial reassessment of the program (17).

Melanocortin-4 receptor agonists

Leptin stimulates the ARC-located POMC neurons that project from the ARC to other hypothalamic regions such as the paraventricular nucleus (PVN) and lateral hypothalamic area (LHA) and suppress appetite by stimulating the release of the anorexigenic neuropeptides corticotrophin-releasing hormone (CRH) and cocaine-and-amphetamine-regulated transcript (CART) in the paraventricular nucleus (PVN). In addition, it inhibits the release of the orexigenic neuropeptides orexin and melanocortin-concentrating hormone (MCH) in the LHA, through the release of CART and melanocyte-stimulating hormone (α-MSH). The latter derives from the cleavage of POMC by prohormone convertase-1 and acts via melanocortin-3 and -4 receptors (Mc3r, Mc4r) activation. Although α-MSH rose as promising novel antiobesity drug target, it was not eventually proved to induce any significant reduction in body weight (18).

Melanin-concentrating hormone (MCH) antagonists

The melanocortin-concentrating hormone (MCH) suggests another important orexigenic neuropeptide in the LHA. Its release is stimulated by NPY and inhibited by leptin, while it exerts its orexigenic effects through the MCH1 receptor (MCHR1) (19). Similarly to NPY, MCH also exerts pleiotropic functions such as locomotor activity, sensory processing, anxiety, aggression and learning. Thus, despite the role of MCH in hunger stimulation, MCHR1 blockage potential as an antiobesity target is questionable because such inhibition could elicit undesirable side effects. **BMS -830216** (Bristol-Myers Squibb), a pharmacological antagonist of MCH signaling, is in phase I/II clinical testing to evaluate the safety, tolerability and effect of different doses of BMS-830216 on body weight and other obesity-related factors, compared to placebo (20).

Subtype-selective serotonin-receptor agonists

Central serotonin levels participate in feeding behavior and energy balance modulation, and two selective serotonin reuptake inhibitors (SSRIs), fluoxetine and sertraline, induce weight loss in obese subjects. The ARC located NPY neurons release agouti-related protein (AgRP) and gamma-aminobutyric acid (GABA) both tonically inhibit the anorexigenic POMC neurons. Further, leptin and insulin inhibit the NPY/AgRP neurons thus decrease the GABAergic inhibitory input to POMC cells, and it was shown that the serotonin (5-HT) system also directly modulates the hypothalamic POMC and NPY networks enhancing satiety and causing hypophagia. These effects are mediated by 5-HT2C and 5-HT1B receptors, located on hypothalamic POMC and NPY neurons, respectively. It was demonstrated that through the

5-HT1B receptors serotonin hyperpolarizes and inhibits the NPY/Agrp neurons, decreasing the GABAergic inhibitory input to POMC cells while it also activates the POMC neurons through its effects on 5-HT2C receptors. By these actions, serotonin increases α-MSH and decreases AgRP release at the hypothalamic melanocortin system, promoting satiety. As activation of the 5-HT1B receptor has been implicated in both primary pulmonary hypertension (21) and valvulopathy (22) the 5-HT2C receptor subtype has been implicated as a target for therapeutic intervention. Among several potent and selective 5-HT2C receptor agonists proved to be effective in suppressing food intake and inducing weight loss in rodents such as WAY-163909 (23), CP-809,101 (24) and vabicaserin (25), only **lorcaserin** (APD356) (26) has moved into clinical testing. The Lorcaserin Phase III program [BLOSSOM (Behavioral modification and LOrcaserin Second Study for Obesity Management), BLOOM (Behavioral modification and Lorcaserin for Overweight and Obesity Management), and BLOOM-DM (Diabetes Management)] showed that 47.2% of patients treated with lorcaserin 10 mg twice daily lost at least 5% of their body weight, compared to 25% for placebo. The average weight loss achieved with lorcaserin after a year was 5.9% (12.7 pounds), compared to 2.8% (6.3 pounds) with placebo (27). The FDA however, has declined to approve its application stating that the weight loss efficacy of lorcaserin in overweight and obese individuals without type 2 diabetes was only marginal plus it has raised some concerns regarding tumor formation in high doses in rats (28). A specific **AgRP inhibitor** (**TTT-435**) (TransTech Pharma) is currently in phase II clinical trials having shown promising appetite suppressant efficacy (29).

The cannabinoid-1 receptor (CB1) antagonists

Among other neurotransmitter systems the cannabinoid system modulates the hypothalamic melanocortin and NPY feeding networks. It has been shown that administration of cannabinoid-1 receptor (CB1) agonists and antagonists induce hyperphagia and hypophagia, respectively (30). These observations lead to development of rimonabant, a cannabinoid-1 receptor antagonist, for the treatment of obesity which was shown quite effective in promoting weight loss, however (31) increased the incidence of mood-related disorders. As a result, rimonabant was withdrawn from the market and the development of other cannabinoid-1 receptor antagonists for the treatment of obesity has been also discontinued.

Bupropion

Bupropion is a dopamine and norepinephrine -reuptake inhibitor that has been marketed as an anti-depressant and for smoking cessation. Previous animal studies have clearly shown the dose-dependent food consumption-restricting effects of brupopion following its intraperitoneal injection (32). Consequently, the acute effects of dopamine and noradrenaline reuptake inhibition on energy homeostasis were determined demonstrating their additive effects on short-term food intake (33,34). These findings suggest that the dual role of bupropion as dopamine and noradrenalin-reuptake inhibitor mediates bupropion's centrally appetite-suppressing actions; its effects on appetite have been specifically linked to POMC neuronal signaling activation (35). Whether the acute meal terminating effects of bupropion documented in animal studies could be translated into long-term weight loss efficacy in humans was addressed by three clinical trials with overweight and obese adults (36-38) using different treatment doses (100 to 400 mg/d) and duration (up to 24 weeks). They all have shown the modest weight

reducing efficacy of bupropion plus its safe profile; the documented weight loss was dose dependent. One study that assessed the anti-obesity efficacy of bupropion over 2 years reported maintenance of the weight loss during the continuation phase (38) while another one proved its efficacy even in depressed patients (37). Although the weight loss effect of bupropion was superior in non-depressed patients compared to those suffering from depression, the fact that bupropion was well tolerated and effective in this particular group of patients provides a potential valuable adjunctive therapy to elevate mood in depressed subjects in who weight gain secondary to anti-depressant therapy is an issue. Cardiovascular effects, such as a rise in blood pressure and tachycardia were usually mild, and the risk of seizure, which was high with the original bupropion formulation, has been significantly reduced with the advent of bupropion-SR and bupropion-ER.

An interesting finding of the previous studies was that the only modest weight loss effect of bupropion reached a plateau by 24 weeks of treatment (36,37). This could be explained by the molecular pathophysiology of the weight reducing effects of bupropion which directly stimulates the hypothalamic POMC neurons that in turn release α-MSH and β-endorphin. α-MSH mediates the anorectic effect of POMC activation, whereas β-endorphin exerts negative feedback via opioid receptors on POMC neurons (39); the latter could suggest one of the compensatory mechanisms that limits long-term efficacy of bupropion and other weight loss modalities. Addressing this hypothesis, opioid antagonists were shown to decrease short-term food intake (40), perhaps by blocking β-endorphin. They failed however to produce consistent or clinically meaningful weight loss, when the opioid antagonist naltrexone was tested even at large doses (300 mg/d) (41-43) suggesting that a single opioid mechanism is unlikely to explain all aspects of ingestive behavior. Based on these findings, the weight loss efficacy of the combined **bupropion/naltrexone** (**Contrave**) therapy was recently compared to monotherapy and placebo by several studies. The Contrave Obesity Research I study (phase 3 trial) reported that the sustained-release combination of naltrexone/bupropion could be a useful therapeutic option for treatment of obesity along with lifestyle modifications (44). Similar were the findings of another study that confirmed the efficacy and safety of naltrexone/bupropion combination as an adjunct to lifestyle and behavior modification (45). Both studies documented sustained weight loss for the time course studied (max 56 wk trial). FDA however recently rejected Contrave due to concerns about the heart side effects of the drug.

Zonisamide

Having explained the pathophysiology behind the anti-obesity efficacy of the selective serotonin-receptor agonists and the dopamine-reuptake inhibitors, it would have been ideal to have a drug that could combine serotonergic and dopaminergic activity. This is exactly what happens with **zonisamide** , a marketed antiepileptic drug that exerts dose-dependent biphasic dopaminergic (46) and serotonergic (47) activity. Its weight loss efficacy was addressed by a double-blind, placebo-controlled trial which reported 32-week mean weight loss of 9.2 kg (1.7 kg) (9.4% loss) for the zonisamide group (used dose up to 600 mg/d) compared with 1.5 kg (0.7 kg) (1.8% loss) for the placebo group (P<.001). Zonisamide was generally well tolerated, with only few adverse effects (48). Similar were the findings when the long-term effectiveness and tolerability of zonisamide for weight control was examined in psychiatric outpatients using various psychotropic medications; the mean BMI reduction achieved was 0.8±1.7 kg/m2 and ranged

from -2.9 kg/m2 to 4.7 kg/m2 ($p<0.001$) while the drug was generally safe and well tolerated (49). Zonisamide was also assessed in the treatment of binge-eating disorder where it was proved to be effective in reducing binge-eating frequency, severity of illness, and weight. However, the reports regarding its tolerability were conflicting (50,51).

Whether the anti-obesity efficacy of zonisamide is increased when it is combined with bupropion (dopamine and norepinephrine -reuptake inhibitor) has been evaluated in a few Phase II clinical trials; the **bupropion/zonisamide** combination is under the trade name **Empatic** . In a 12-week pilot study of 18 obese women, the reported body weight loss was 2.9 kg (3.1%) in the zonisamide group, significantly less than the 7.2 kg (7.5%) noted in the combination-therapy group (zonisamide 100 mg rising to 400 mg over 4 weeks and bupropion 100 mg rising to 200 mg after 2 weeks) (52). Interestingly, the tolerability of the combination was better than that of zonisamide monotherapy. Finally, in another 24-week double-blind placebo-controlled Phase IIb trial (53) zonisamide 120 mg and 360 mg monotherapy reduced body weight by 3.2% and 5.3%, respectively, while bupropion 360 mg reducedby 2.3% and placebo by 1.4%. On the other hand, Empatic containing 120 mg zonisamide/360 mg bupropion resulted in a 6.1% weight loss, while the 360 mg zonisamide/ 360 mg bupropion combination resulted in 7.5% reduction of body weight. These reports suggest significant weight-reduction effect for the combination bupropion/zonisamide. However, several concerns regarding bupropion (54,55) and zonisamide (56,57) safety need to be addressed in upcoming Phase III studies for Empatic before firm conclusions about its safety profile can be drawn.

Topiramate

Topiramate is another anticonvulsant agent associated with weight loss (58). It is a sulphamate-substituted fructose that is approved as an anti-epileptic/antimigraine agent and which has multifactorial effects on the CNS, including action on the orexigenic GABA (γ-amino butyric acid) systems causing appetite suppression (59). A 6-month dose-ranging study in obese human subjects addressing its anti-obesity efficacy at doses of 64, 96, 192, and 384 mg/day (in divided twice-daily dosing) concluded that all doses produced significantly greater weight loss compared to placebo, and that weight loss in the 192 mg/day group was similar to the 384 mg/day group (60). This is important as topiramate has been associated with several neuropsychiatric effects, especially when administered at high doses (of 192 mg/d or more). Another study investigating the weight loss efficacy and safety of topiramate doses of 96, 192, and 256 mg/day over a 1-year period in obese subjects using the immediate release form tablets (before the development of the controlled-release formulation with the potential to enhance tolerability and simplify dosing) reported clinically significant weight loss (loss of 7.0, 9.1, and 9.7% of their baseline body weight for the dose of 96, 192, and 256 mg/day, respectively, compared to 1.7% body weight loss in the placebo group; $P<0.001$) plus improvements in blood pressure and glucose tolerance (61). Finally, some other studies investigated the therapeutic effect of topiramate in patients with binge eating disorder (62) and bulimia (63) that are both associated with obesity; the results were very promising regarding control of symptoms in both disorders.

As mentioned before, the higher the dose of topiramate (especially in doses equal to or higher than 192 mg/d) the more the associated adverse effects including suicidality, metabolic

acidosis, acute myopia and secondary angle closure glaucoma. Therefore a lower dose of topiramate was used (in a special controlled release formulation) in a novel anti-obesity drug called Qnexa in combination with phentermine, an amphetamine derivative which has central anorectic effects and is still prescribed in the USA for the short-term treatment of obesity. The weight loss efficacy of the new appetite suppressant drug (orally administered) was estimated over a 1-year trial (CONQUER study; Phase III study) testing different doses of each component (64); once-daily phentermine 7·5 mg plus topiramate 46·0 mg, or once-daily phentermine 15·0 mg plus topiramate 92·0 mg. According to the study the weight loss effect of **Qnexa (topiramate/phentermine)** was dose-depended; the average weight loss for patients on the higher dose of Qnexa was 14.4% of body weight, compared to 6.7% among patients on the lower dose and 2.1% among patients taking a placebo. In addition, it was shown to improve various cardiovascular aspects as well as a sleep apnea. However, 60% of patients treated with the highest dose of the experimental combination drug dropped out of the study early because of the side effects, compared to 11% of patients in the lower-dose group and 8.4% of people who got the placebo treatments. At least two other Phase III studies have been completed, testing two different dose combinations of phentermine and topiramate, the EQUATE (756 obese subjects comparing mid- and full-dose Qnexa with the individual components and a placebo over 28 weeks) and the EQUIP (1267 morbidly obese patients treated with low- or full-dose Qnexa or placebo over 52 weeks) (65,66). They both confirmed the anti-obesity efficacy of Qnexa. It was however revealed that the rate of withdrawal of patients on the highest dose for depression, anxiety and sleep disorders was sevenfold higher than in the placebo group while the highest dose combination was also associated with an increased heart rate of 1.5 bpm, which was not seen with the mid-dose combination, and slightly more disturbances in attention, amnesia and memory impairment. Because of these findings, FDA rejected Qnexa a year ago over (67) expressing concerns over the risk of psychiatric and cognitive dysfunction and about potential heart problems and birth defects in babies born to women who take the drug (a 20-fold increase in cleft palates), agreed though to let the company resubmit its application for limited approval for use in adults who are not likely to become pregnant . If this happens, Qnexa would be the only prescription appetite suppressant approved for long-term use available in the U.S.A.

Neuropeptide Y (NPY) inhibitors

The ARC NPY neurons inhibit the anorexigenic POMC neurons (via NPY Y1 and Y5 receptors) and promote the release of the orexigenic neuropeptides orexin and MCH in the LHA, thus promoting food intake (68). Therefore, NPY blockage could suggest a promising target for the body weight management. Animal experiments (in mice) have shown that pharmacologic blockade or genetic deletion of the Y1- and Y5-receptors reduces food intake and weight, with Y1-receptor signaling appearing to be the major mediator of the orexigenic effects of NPY. However, NPY is the most abundant central neuropeptide and regulates many functions beyond feeding; thus targeting NPY neurons/Y receptors particularly for obesity is not easy, and could result in unacceptable side effects. In addition, experimental medical blockade of NPY signaling with the **Y5-receptor antagonist MK-0577** failed to cause any significant weight loss in a 1-year clinical trial (69). On the other hand, the **selective Y5-receptor antagonist S-2367** (Shionogi USA, Inc.) induced a mean placebo-adjusted weight loss of 3.0% baseline weight ($p<0.0001$) over 54 weeks of therapy (70). Putting together, Y5-receptor antagonists seem to

attain limited efficacy regarding weight loss, however the combined Y1/Y5-receptor antagonism may prove more effective. We are not aware of any Y1/Y5-receptor antagonist in development up to date. In contrast to Y1 and Y5, the Y2- and Y4-receptors are the targets of the satiety hormones PYY and pancreatic polypeptide, respectively, and as it is mentioned below two drugs a **Y2/Y4-receptor agonist (obinepitide;** 7TM Pharma) and a **selective Y4-receptor agonist (TM30339** ; 7TM Pharma), are in phase I/II clinical trials having results that are quite promising regarding weight loss.

GASTROINTESTINAL AND PANCREATIC PEPTIDES THAT REGULATE FOOD INTAKE

The gut-brain axis plays important role in food consumption regulation. During food intake information regarding meal quality and content and short-term alterations in nutrient status is relayed from the gastrointestinal (GI) tract and pancreas to the brain which in turn determines meals size. Further to feeding, a number of satiation signals optimize these processes by influencing gastrointestinal motility and secretion. Several peptides have been recognized to mediate this GI system-brain communication including satiety signals such as gastrin releasing peptide (GRP), cholescystokinin (CCK), peptide YY (PYY), glucagon-like peptide-1 (GLP-1), pancreatic polypeptide, glucagon and amylin, and the orexigenic peptide ghrelin. While the anorexigenic peptides are secreting during feeding, ghrelin is secreted before meals and acts to increase hunger and meal initiation. Some of the GI and pancreatic peptides implicated in the regulation of food intake act directly to regions of the brain involved in the regulation of food intake, including ARC in hypothalamus and the area postrema, while others act outside of the CNS, for example modulating the activity of neurons such as the vagal nerve, which projects to the nucleus of the solitary tract in the brain stem.

Upper-intestinal satiation:

CCK and CCK1R agonists

CCK is the first described intestinal satiation peptide (71); it is produced by the mucosal I cells of the duodenum and jejunum, and the enteric nervous system in response to luminal nutrients especially lipids and proteins. Through endocrine and/or neural mechanisms, CCK regulates many GI functions, including satiation, by acting on two CCK-specific receptors. The CCK receptor 1 (CCK1R) is expressed mainly in the GI system, and the CCK2R predominates in the brain. The vagus nerve plays a critical role for the CCK-induced satiation as it has CCK1R (72,73) and suggests the afferent pathway through which CCK relays satiation signals from the GI to the hindbrain region (74,75). Supporting this is the well documented attenuation of the CCK-induced satiation following abdominal subdiaphragmatic vagotomy (76-78). In addition, CCK inhibits gastric emptying, thereby augmenting gastric distention and mechanoreceptor stimulation which in turn augments the anorectic effects of CCK (79-81). Remarkably, a negative feedback mechanism of blood lipids on circulating CCK might exist (82). This could suggest lipid lowering treatment may have the potential to restore physiological CCK action. Despite the satiety effect of CCK, its potential as an antiobesity target is questionable. Human

studies with intravenously infused **CCK carboxy-terminal octapeptide** (CCK-8) have shown decreases in meal size and duration in a dose-dependent manner (83-86). However the CCK satiating effects were very short-lived and usually did not last more than 30 minutes raising issues regarding its importance in long-term body-weight regulation. In an animal study, chronic CCK administration with up to 20 peripheral injections per day, reduced meal size was associated with increased meal frequency, leaving body weight unaffected (87). Finally, disappointing were the reports from trials testing **CCK 1R agonists** as potential antiobesity drugs (88). It is currently suggested that there might be role for CCK in body-weight regulation not as monotherapy but maybe as adjunctive/synergistic therapy to long-term adiposity signals, such as leptin (89,90).

Lower-intestinal satiation:

Glucagon-like peptide-1 analogues

The dominant role of GI in satiation (15,91) is mediated not only by the gastric mechanoreceptors and upper intestinal neuropeptides such as CCK, but also by gut satiation peptides that are secreted from lower-intestine enteroendocrine cells in response to ingested food. They in turn diffuse through interstitial fluids to activate nearby nerve fibers and/or enter the bloodstream to function as hormones and augment the perception of GI fullness by acting in specific parts of the CNS. There is a well defined duodenal-ileal communication (ileal brake) by which proximal intestine informs distal intestine regarding meal quality and content so that the latter modulates/restricts feeding duration, proximal GI motility and gastric emptying, and also regulates metabolic responses to nutrient intake. Glucagon-like peptide-1 (GLP-1) suggests such a mechanical and behavioral brake on eating and is produced primarily by L cells in the distal small intestine and colon. Along with glucagon and oxyntomodulin, GLP-1 is cleaved from proglucagon, which is expressed in the gut, pancreas, and brain. The GLP-1 equipotent bioactive forms GLP17–36 and GLP17–37 are rapidly inactivated in the circulation by dipeptidyl peptidase-4 (DPP4). Therefore GLP-1 analogues that have slightly different molecular structure but significantly longer duration of action compared to the wild GLP-1, have been used for therapeutic interventions in patients with diabetes in whom they significantly improved glycemic control, fasting plasma glucose, beta cell function and probably β-cell regeneration. Currently, the GLP-1 analogues used in clinical practice for diabetes control are Exenatide and Liraglutide. Beyond improved glycemic control, clinical studies have also demonstrated anorectic effects and significant weight loss by these agents (92-97). Although the exact mechanisms by which GLP1 induces anorexia are not fully known, it is suggested that vagal and possibly direct central pathways are involved (98,99). The GLP-1 receptor R (GLP1R) is the principle mediator of the anorectic effects of GLP-1 (100) and is expressed by the gut, pancreas, brainstem, hypothalamus, and vagal-afferent nerves (101).

The **GLP-1 analogues** are not currently approved for obesity treatment, however their impressive effects on body weight in diabetic patients led to a two-year randomized trial of liraglutide (Victoza, Novo Nordisk) addressing this indication. The participants were nondiabetic subjects and liraglutide was compared to the weight-loss drug orlistat (Xenical, Roche) (102); the drugs were taken in combination with diet and exercise recommendations. At two years,

mean weight loss among all 268 subjects who completed the study was greater in patients taking the 2.4-mg or 3.0-mg dose of liraglutide, as compared with those taking orlistat (7.8 kg vs 5.4 kg), although this difference missed statistical significance (p=0.09). However, patients randomized to liraglutide for the duration of the study had lost a mean of 10.3 kg, as compared with 6.7 kg in the orlistat group while more patients taking liraglutide, as compared with orlistat, had lost more than 5% of their weight at two years (69% vs 49%; p<0.03). Liraglutide is not yet licensed as anti-obesity drug. The fact that doses of liraglutide higher than those used for the treatment of diabetes are need for weight loss (2.4-mg to 3.0-mg vs 1,2 to 1,8 mg) is slightly worrisome in the light of slightly increased incidence of medullary thyroid tumors in two nonhuman species in clinically relevant liraglutide doses (103). The absence of alternative effective anti-obesity drugs in current clinical practice makes GLP1R overstimulation a quite attractive pharmacologic antiobesity target, as it reduces body weight while independently ameliorating diabetes. Therefore, some other long-acting GLP-1 analogs are currently investigated for diabetes treatment but also for weight loss. Once daily 13-week treatment with 20 μg or 30 μg of **lixisenatide** (by Zealand Pharma) reduced body weight significantly more compared to placebo (-3 kg for lixisenatide 20 μg; p<.01, -3.47 kg for lixisenatide 30 μg; p<.01, -1,94 kg for placebo) (104). Current findings regarding **CJC-1134-PC** (from ConjuChem Biotechnologies Inc.), which is a conjugate of exendin-4 and recombinant human albumin and represents a once-weekly glucagon-like peptide-1 receptor agonists, suggest that it provides similar reduction in body weight compared with exenatide twice daily but may have more favorable adverse event profile, which might improve the treated patients compliance and probably total weight loss in the long term (105). Finally, **albiglutide** (by GSK) and **taspoglutide** (by Roche) two novel GLP-1 analogues are currently investigated. A recent review examined the efficacy, safety and perspective for the future of the once-weekly GLP-1 receptor agonists exenatide once weekly, taspoglutide, albiglutide, **LY2189265** and CJC-1134-PC, and compared them to the currently available agonists, exenatide BID and liraglutide QD. It concluded that the long-acting agonists are not superior compared to currently used exenatide BID and liraglutide QD regarding weight loss (106).

In a separate development, Emisphere has formulated orally administered **PYY3–36 and GLP-1 combination** using a sodium *N* -[8-(2-hydroxybenzoyl) amino] caprylate (SNAC) carrier (107). Early studies revealed that the neuropeptides delivered orally in this way have a pharmacodynamic profile consistent with reported pharmacology, were rapidly absorbed from the gastrointestinal tract and reached concentrations several-fold higher than those seen naturally postprandially (108,109). The oral GLP-1 (2-mg tablet) alone and the combination of oral GLP-1 (2-mg tablet) plus PYY3–36 (1-mg tablet) showed enhanced fullness at meal onset and induced a significant reduction in energy intake (107).

PYY

PYY is a 36–amino acid peptide (PYY1–36), member of the pancreatic polypeptide–fold (PP-fold) family which also includes PYY, NPY, and PP, and interacts with a family of receptors (mainly Y2R). It is produced postprandially in response and proportionally to caloric load, by the distal-intestinal L cells along with oxyntomodulin (OXM) and GLP-1. Just like GLP-1 and OXM, PYY1–36 is rapidly proteolyzed by DPP4. However, unlike the other two neuropeptides, the cleaved product PYY3–36, is bioactive. Human studies have shown that PYY delays gastric

emptying, and promotes satiety (110), while short-term intravenous administration of PYY3-36 at doses generating physiologic postprandial blood excursions was shown to decrease calorie intake by approximately 30% in lean and obese subjects, without causing nausea, affecting food palatability, or altering fluid intake or followed by compensatory hyperphagia (111). Another study confirmed the above findings reporting dose-dependent reduction of food intake (maximal inhibition, 35%; P < .001 vs control) and calorie intake (32%; P < .001) after intravenous infusions of several different concentrations of PYY3–36 (112), while Sloth et al. showed for first time the almost significantly higher energy expenditure following PYY3–36 intravenous infusion compared with PYY1–36 or control. In a recent study the effect of infused PYY3–36 on energy intake was compared to that of OXM or the combined **PYY3–36** /**OXM** treatment; the results showed that energy intake was significantly less with the combined treatment compared to PYY3–36 or OXM monotherapy (113). Whether these findings could suggest weight loss efficacy of PYY was evaluated in a 12-week trial of 133 obese patients who were randomly assigned to intranasal PYY3–36 (200 or 600 mcg three times a day before meals) or placebo, in conjunction with diet and exercise. In the dose of 200 mcg PYY3–36 failed to reduce body weight while 60% of patients treated with the high PYY3–36 dose (600 mcg three times a day) dropped out due to nausea and vomiting so that no meaningful inference could be drawn from the few patients who completed the study on 600 μg (114). These findings are in contrast to what happens in rodents (115-121) and nonhuman primates (122), where PYY3–36 preparations reduce body weight. One suggested explanation is that the PYY3–36 effect is critically modulated by the time of injection. As the main antiorexigenic effect of PYY is by Y2R-mediated NPY inhibition, PYY is obviously more effective at times that the orexigenic NPY is increased. In accordance with this theory is the reported weight loss effect of PYY3–36 when injected in rodents at fasting state or in the early dark cycle — times when NPY is naturally induced (123).

PYY3–36 is structurally similar to pancreatic polypeptide (PP); PYY3–36 acts mainly through Y2R while PP through Y4R. A synthetic analog (**TM30338) (obinepitide** ; 7TM Pharma) that stimulates both Y2/Y4-receptors has been developed. Recently, reported data suggest that once-a-day subcutaneous dosing of obinepitide in obese human subjects inhibited food intake, at a statistically significant level, up to at least nine hours after dosing (124).

Oxyntomodulin

Oxyntomodulin (OXM) is a 37-amino acid anorexigenic peptide hormone produced in the L-cells of the distal small intestine and colon, where it colocalizes with GLP-1 and PYY. Animal studies have shown weight reduction (and improved glucose metabolism) following chronic OXM injections beyond that explained by food intake restriction, suggesting additional effect of OXM on energy expenditure (125-126). Just like GLP1, OXM is a proglucagon gene product and is believed to modulate the energy homeostasis system at least in part via GLP1R although its GLP1R binding affinity is about 100 times lower than that of GLP1 (125,127). Centrally however, GLP1 and OXM have different targets as OXM activates neurons in the hypothalamus (126), whereas GLP1 does so in the hindbrain and other autonomic control areas (128). In human studies, intravenously infused OXM acutely act as an endogenous satiety signal and reduced appetite and food intake (129) while in a 4-week trial with OXM injections three times a day 30 minutes before meals in a group of overweight and obese volunteers (n = 14) it resulted

in reduced nutrient intake (35% ± 9%) associated with significant weight loss compared to placebo (2.3 ± 0.4 kg vs 0.5 ± 0.5 kg, respectively) (130). Similar were the findings of another study with twelve overweight or obese human volunteers who underwent a randomized, double-blinded, placebo-controlled study. An ad libitum test meal was used to measure energy intake during intravenous infusions of either PYY3–36 or OXM or **combined PYY3–36 /OXM** . Again, OXM significantly reduced energy intake compared to placebo, although the combined treatment had superior effects compared to PYY3–36 or OXM monotherapy (113). Human studies have also clearly demonstrated the direct effect of OXM on energy expenditure (131). These modest but favorable results suggest significant promise for OXM-based therapies for obesity. However, as for other peptide hormones, its clinical application is limited by the short circulatory half-life, a major component of which is cleavage by DPP-IV. Therefore, structurally modified analogues with altered OXM pharmacological profile have been produced with longer duration of action and good safety profile and effects on body weight (and glucose metabolism) management in animal studies (132-133) bringing closer their usage in human clinical trials. Furthermore, the crystal structure of oxyntomodulin has been solved, and this advance should facilitate the rational design of oxyntomodulin peptidomimetics to be tested as oral antiobesity pharmaceuticals. Immediate future will reveal OXM role in obesity management.

Ghrelin vaccines and Ghrelin inhibitors

Ghrelin, is a 28 amino acid peptide produced primarily by the stomach and proximal small intestine (134). It is the only known circulating orexigenic hormone and signals both on vagal afferents and in the arcuate nucleus where it powerfully enhances NPY orexigenic signaling (135-136). Its levels increase before meals and get suppressed by the ingested nutrients with carbohydrates being the most effective ones (compared to proteins and lipids). Its suppression results from neurally transmitted (nonvagal) intestinal signals, augmented by insulin. An experimental **ghrelin vaccine, CYT009-GhrQb** , was discontinued in 2006 as it didn't have the expected effects on weight loss but a novel one conjugated to the hapten, keyhole limpet hemocyanin (KLH), tested in rodent models was shown to decrease feeding and induce weight loss (137). Furthermore, in 2006, NOXXON Pharma AG granted Pfizer an exclusive worldwide license to **NOX -B11** , a ghrelin-neutralizing RNA spiegelmer, that attaches to the active form of ghrelin and blocks its ability to bind to its receptor, and thus to block the orexigenic activity of exogenously administrated ghrelin in rats (138). However, NOX-B11 did not affect basal food intake in nonfood-deprived rats. This treatment may only be efficacious when plasma ghrelin levels are high, such as before a meal or during times of food restriction (dieting).

Since the discovery that the effects of ghrelin are primarily mediated by the GH secretagogue receptor **(GHSR) 1a** , there have been multiple potent, selective, and orally bioavailable ghrelin antagonists produced with good pharmacokinetic (PK) profiles that are currently in preclinical testing. An amide derivative 13d (Ca^{2+} flux IC50 = 188 nM, [brain]/[plasma] = 0.97 @ 8 h in rat) for example, showed a 10% decrease in 24 h food intake in rats, and over 5% body weight reduction after 14-day oral treatment in diet-induced obese (DIO) mice (139). Moreover, the discovery of ghrelin O-acyltransferase (GOAT) as the enzyme that catalyzes ghrelin octanoylation and confers its biological activity, revealed several therapeutical possibilities including the design of drugs that inhibit GOAT and block the attachment of the octanoyl group to the ghrelin third serine residue. Such **GOAT inhibitors** could potentially prevent or treat

obesity (140).

Fat-specific satiation peptides:

Enterostatin and apolipoprotein A-IV

Enterostatin and apolipoprotein A-IV suggest GI peptides that are specifically stimulated by fat ingestion and subsequently regulate intake and/or metabolism of lipids. Although peripheral and central enterostatin administration decreases dietary fat intake in animals (and enterostatin-receptor antagonists did the opposite) (141), its administration to humans has thus far shown no effects on food intake, appetite, energy expenditure, or body weight (142). Similarly, apolipoprotein A-IV which is synthesized and secreted exclusively by the small intestine (primarily by the jejunum, but also by the duodenum and ileum) acts as a satiety factor that is downregulated by leptin (143,144) and upregulated (synthesis and secretion) by insulin and PYY in both rodents and humans (145,146). Although exogenous administration of apolipoprotein A-IV was quite effective regarding meal size, food intake, and weight gain reduction in rats (147,148), we still lack data regarding apo A-IV therapeutic administration in humans and its effects on body weight.

Pancreatic satiation peptides:

Pancreatic polypeptide (PP)

Pancreatic polypeptide (PP), is a 36-amino-acid peptide which is structurally similar to PYY. It is primarily produced in the pancreas in response to ingestion of food and in proportion to caloric load (149). Animal studies have shown that peripheral administration of PP decreases feeding (through Y4R in the area postrema), whereas centrally administrated PP increases it (through Y5R deeper in the brain) (150). In humans, intravenous infusion of PP (10 pmol/kg/min) (supra-physiological levels of PP) in ten healthy volunteers (men and women of normal body weight) caused a sustained decrease in both appetite and cumulative 24-hour energy intake by 25.3 +/- 5.8% (151). Similar were the findings of another study addressing the anorexigenic effect of a lower infusion rate of PP (5 pmol/kg/min) in lean fasted volunteers, holding promises for potential use as anti-obesity agent (152). Another trial studying whether combined treatment with PP/PYY3-36 is superior regarding weight loss compared to either agent alone, concluded that PP and PYY3-36 do not inhibit feeding additively in humans (153); again, this study was hold on lean subjects. In the opposite, as previously mentioned, a synthetic analogue (TM30338) of both PYY3–36 and PP which acts as an agonist of both the Y2 and Y4 receptors holds very promising results regarding early meal termination when administered once-a-day subcutaneously in obese human subjects (124). Similarly, primarily reports of a **selective Y4-receptor agonist (TM30339** ; 7TM Pharma) currently under development were also quite promising regarding effect on reduction of food intake and weight loss.

Amylin and amylin analogues

Amylin, is a 37-amino acid neuroendocrine peptide hormone cosecreted postprandially with

insulin by pancreatic β cells. Among other properties, amylin has centrally mediated glucoregulatory and anorexigenicic actions (154) inhibiting gastric emptying and glucagon secretion as well as decreasing meal size and calorie intake (fat specific) (155) in a dose-dependent manner. These are vagus-independent actions and are exerted via binding to specific amylin receptors in the hindbrain area postrema (156,157) which is in contrast to the peripheral neural mechanisms engaged by most other gut peptides involved in energy homeostasis system regulation. The anorectic efficacy of amylin along its glucoregulatory actions were investigated in human studies with the usage of **pramlintide** which is administered by subcutaneous injections. The latter is a stable, soluble, non-aggregating, equipotent amylin analog that retains a broad range of the pharmacodynamic and pharmacokinetic profile of the native hormone, including receptor binding, and differs from amylin by only three amino acids (158,159). Studies in patients with type 1 and type 2 diabetes have shown great improvement in glycemic control plus sustained reductions in food intake and meal size, as well as mild progressive weight loss following acute and long-term adjunctive pramlintide treatment (120 μg) (160-162); the most common adverse event associated with pramlintide use was transient, mild-to-moderate nausea. This weight loss is noteworthy because it occurred in subjects with type 2 diabetes, upon concomitant insulin therapy, and in the face of a significant A1C reduction, factors that all favor weight gain. Similar to the GLP-1 analogues discussed previously, pramlintide is currently approved for the treatment of type 1 and type 2 diabetes. Whether pramlintide could be a potent anti-obesity agent was investigated in well designed trials addressing this issue. In such a study (16 week randomized, double-blind, placebo-controlled) 204 obese non-diabetic individuals were treated with self-administered subcutaneous injections of pramlintide (nonforced dose escalation ≤ 240 μg) or placebo three times a day 15 minutes before meals without concomitant lifestyle intervention (163). According to the results, pramlintide was generally well tolerated and approximately 90% of the pramlintide-treated subjects were able to escalate to the highest dose of 240 μg three times a day. In contrast to the placebo-treated subjects who experienced minimal changes in body weight over the 16-wk treatment period, the pramlintide-treated subjects had significant weight loss from baseline as early as wk 2, which was progressive up to wk 16, with no evidence of a plateau. At wk 16, the placebo-corrected reduction in body weight after pramlintide treatment was statistically significant compared with placebo (3.7 ± 0.5%, $P < 0.001$; 3.6 ± 0.6 kg, $P < 0.001$). Furthermore the reduction in weight in pramlintide-treated subjects was accompanied by a significant reduction in waist circumference compared with placebo-treated subjects after 16 wk of treatment (evaluable 4.3 ± 0.6 vs. 0.7 ± 0.9 cm, $P < 0.01$). At the end of the 16 week trial, 31% of the subjects treated with pramlitide achieved ≥5% weight loss compared to just 2% of the placebo group ($P < 0.001$). Interestingly, 8 weeks after treatment cessation, the pramlintide-treated subjects had on average regained one third of the overall weight loss observed by wk 16. These findings constitute a proof of concept that pramlintide may have therapeutic use as an antiobesity agent. Remarkably, at this higher dose (240 μg three times a day), the mean reduction in body weight with pramlintide treatment over 16 wk was approximately twice that previously observed over a similar time frame in insulin-treated subjects with type 2 diabetes who were treated with lower pramlintide doses (120 μg). This might suggest that higher doses of pramlintide might be necessary to achieve significant weight loss, although it is not clear yet whether concurrent insulin treatment was the main cause of that difference.

Previous animal studies have shown that amylin treatment significantly enhanced the

hypothalamic anorexigenic leptin signaling (164) while the combination treatment with amylin and leptin led to marked, synergistic reductions in food intake (up to 45%) and fat-specific weight loss (up to 15%) (164,165). Recently, the weight-lowering effect of the amylin/leptin combination was evaluated in obese human subjects, with the usage of their analogues **pramlintide/metreleptin** , respectively. As previously discussed (see *leptin* paragraph) two trials (15,16) addressing the weight loss efficacy of the combined treatment over 20, 28 and 52 weeks, respectively) reported sustained and robust weight loss by the combined treatment. However, only recently, Amylin Pharmaceuticals, Inc. and Takeda Pharmaceutical Company Limited announced the discontinuation of the combination treatment development following commercial reassessment of the program (17). Amylin Pharmaceuticals, Inc. has also completed a Phase 2 study of **davalintide** , a second-generation analog of amylin, for the treatment of obesity. In this study however, the weight loss efficacy and tolerability profile of davalintide was not superior to pramlintide, and was inferior to the pramlintide/metreleptin combination making Amylin and Takeda, as part of their co-development and commercialization agreement, to halt further development of davalintide (17). Putting together, studying peptide hormones in combination as integrated neurohormonal approaches may hold promise for the effective treatment of obesity. In this direction the anti-obesity effect of the combined treatment **amylin/PYY3–36** was estimated in an animal study, as they both suggest short-term signals of meal termination with anorexigenic and weight-reducing effects (166). Statistical analyses revealed that food intake suppression with the combined treatment was synergistic, whereas body weight reduction was additive; this particular combination has not been studied in humans yet.

Preclinical evidence suggests that pharmacotherapy for obesity using combinations of agents targeting distinct regulatory pathways of the energy homeostasis system may produce robust additive or synergistic effects on weight loss. In this direction the safety and efficacy of the combined treatment with **pramlintide/phentermine** and **pramlintide/sibutramine** was evaluated in a randomized placebo-controlled study with 244 obese or overweight, nondiabetic subjects (167). The participants received placebo subcutaneously (sc) t.i.d., pramlintide sc (120 microg t.i.d.), pramlintide sc (120 microg t.i.d.) + oral sibutramine (10 mg q.a.m.), or pramlintide sc (120 microg t.i.d.) + oral phentermine (37.5 mg q.a.m.) for 24 weeks. The results suggest that the weight loss achieved at week 24 with either combination treatment was greater than with pramlintide alone or placebo ($P < 0.001$; 11.1 +/- 1.1% with pramlintide + sibutramine, 11.3 +/- 0.9% with pramlintide + phentermine, -3.7 +/- 0.7% with pramlintide; -2.2 +/- 0.7% with placebo; mean +/- s.e.), without any major adverse events. In view of recent withdrawal of sibutramine from the market due to adverse cardiovascular events and the short period of time licensed usage of phentermine for weight loss, the pramlintide/phentermine combination could be used as short term anti-obesity agent.

PERIPHERAL MODULATORS OF THE EFFICIENCY OF DIGESTION, METABOLISM AND LIPOGENESIS

Lipase inhibitors

In addition to early termination of food intake that is augmented by the centrally acting appetite

suppressants, another potential therapeutic anti-obesity approach is the induction of a negative energy balance through the inhibition of nutrient, particularly fat, absorption. Lipase inhibitors inhibit gastric and pancreatic lipases in the lumen of the gastrointestinal tract to decrease systemic absorption of dietary fat. Orlistat (Xenical; Roche), is currently the only marketed anti-obesity drug of this category licensed for the treatment of obesity (including weight loss and weight maintenance). The only other pancreatic and gastrointestinal lipases inhibitor currently in clinical development is **ATL -962 (Cetilistat** ; Alizyme).

A short-term (12-week) randomized, placebo-controlled study of weight reduction addressing the efficacy, safety and tolerability of cetilistat in obese patients reported that cetilistat produced a clinically and statistically significant weight loss in obese patients to similar extents at all doses examined compared to placebo (60 mg t.i.d. 3.3 kg, $P<0.03$; 120 mg t.i.d. 3.5 kg, $P=0.02$; 240 mg t.i.d. 4.1 kg, $P<0.001$) plus it significantly improved other obesity-related parameters including waist circumference, serum cholesterol and low-density lipoprotein cholesterol levels. Cetilistat treatment was also well tolerated and the common orlistat-induced GI adverse events, such as flatus with discharge and oily spotting, only occurred in 1.8-2.8% of subjects in the cetilistat-treated group (168). The combined results from three phase I clinical studies designed to investigate the efficacy, pharmacodynamics and tolerability of a range of cetilistat doses [50 mg t.i.d. (n = 7), 60 mg t.i.d. (n = 9), 100 mg t.i.d. (n = 7), 120 mg t.i.d. (n = 9), 150 mg t.i.d. (n = 16), 240 mg t.i.d. (n = 9) and 300 mg t.i.d. (n = 9)] compared with placebo or orlistat [120 mg t.i.d. (n = 9)] in healthy volunteers (169) were published. They report that cetilistat is equipotent with orlistat regarding faecal fat excretion, attains though much better tolerance profile as the number of episodes of steatorrhoea per subject in the orlistat group (4.11) was 2.5-fold greater than that of the cetilistat-treated group. The different tolerance profile between the two lipase inhibitors, seems to be related to the physical form of the fat in the intestine (rather than the amount of fat) resulted from each medication. Thus, cetilistat acts more like a detergent, whereas orlistat may promote the coalescence of micelles, leading to oils and increased gastrointestinal adverse events. Finally, a recent 12-week trial compared the efficacy and safety of cetilistat (40, 80, or 120 mg three times daily) and orlistat (120 mg t.i.d.) relative to placebo in obese patients with type 2 diabetes on metformin (170). According to this study, similar reductions in body weight were observed in patients receiving cetilistat (80 or 120 mg t.i.d.) or orlistat; these reductions were significant compared to placebo (3.85 kg, $P = 0.01$; 4.32 kg, $P = 0.0002$; 3.78 kg, $P = 0.008$). Furthermore, treatment with cetilistat (80 or 120 mg t.i.d.) or with orlistat significantly improved glycemic control relative to placebo; again, cetilistat was well tolerated, and showed fewer discontinuations due to adverse events than in the placebo and orlistat groups. Based on these findings, cetilistat suggests a novel lipase inhibitor which is currently the furthest in the clinical development of new drugs of this class.

Growth Hormone (GH) and GH lipolytic domain synthetic analogues

In addition to its growth effects, GH also has significant metabolic properties including lipolysis induction. On the other hand GH dynamics change with increasing adiposity and GH circulating levels and response to stimuli are repressed in obesity (171). Putting together, one could think that GH administration would be a therapeutic option for weight loss and fat mass reduction in obese individuals. However, the bulk of 16 clinical trials on GH administration in obesity indicate little or no beneficial effects of GH treatment on body weight (172). There is a recent report from

an Australia-based biotechnology company, Metabolic Pharmaceuticals Limited, for the development of a modified fragment of amino acids 177–191 of GH (hGH177–191) (**AOD-9604**) that mimics the lipolytic effects of GH without producing growth effects. AOD-9604 however failed to induce significant weight loss in a 24-week trial of 536 subjects (173) and its development as anti-obesity agent was terminated.

β3-Adrenoreceptor Agonists

The β3-adrenergic receptor is expressed in adipocytes; its activation by cognate β-agonists cause lipolysis and increase thermogenesis. Thyroid hormones increase thermogenesis via thyroid hormone receptor β subtype. However up to now, every trial to develop selective thyroid hormone receptor agonists which are effective in adipose tissue without systemic side-effects has failed. In 2000, a **selective human β3-agonist, L-796568** , was developed (174). Although its acute (4-hr period) administration in overweight human subjects was associated with significant increase in energy expenditure (by ~8%) (175), a 28-day clinical trial investigating the efficacy of chronic use of L-796568 in overweight and obese non-diabetic men receiving the drug (350 mg/d) failed to display any significant changes in body composition or 24-hour energy expenditure (176). The cause for the ineffectiveness of β3-adrenreceptor activation to induce significant and sustained lipolysis in humans may be explained by the fact that human white adipose tissue expresses minimal levels of β3-adrenoreceptors; similarly their expression is also low within the human brown adipose tissue. Eli Lilly is currently sponsoring a phase 2 weight loss efficacy study to address the effectiveness of the **β3-agonist LY-377604 combined with sibutramine** in inducing weight loss in overweight/obese men and women.

11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitors

Previous studies have shown enhanced conversion of inactive cortisone to active cortisol through the expression of 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) in cultured omental adipose stromal cells (177). The autocrine action of cortisol may be crucial in the pathogenesis of central obesity and features of the metabolic syndrome such as insulin resistance. The reports regarding effectiveness of carbenoxolone (nonselective 11β-HSD inhibitor) in reducing central obesity are conflicting (178,179). Currently, several pharmaceutical companies develop selective 11β-HSD1 inhibitors which are effective in adipose tissue and may be more efficacious in improving insulin sensitivity and reducing weight. Preliminary data from animal studies estimating the weight loss efficacy of **T-BVT,** a new 11β-HSD1 pharmacological inhibitor that homes specifically to the white adipose tissue, are very promising regarding its anti-obesity efficacy and amelioration of multiple metabolic syndrome parameters (180).

Angiogenesis Inhibitors

Increasing adiposity is associated with expansion of the adipose capillary bed. Several vascular growth factors are produced by the enlarging adipocytes, for example vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and angiogenin which may in turn facilitate the expansion of the adipose tissue. Thus anti-angiogenesis may be part of the therapeutic approach for the management of body weight. This hypothesis is strengthened by studies where the experimental administration of anti-angiogenic agents in mice from different

obesity models resulted in significant weight reduction and adipose tissue loss (181,182). Remarkably, adaptations in food intake, metabolic rate, and preferred energy substrate were also noticed. These findings may suggest fat tissue modulation by the altered vasculature and underlie the regulatory effect of angiogenesis on physiological processes in adipogenesis. Although there are a large number of foods and beverages containing naturally occurring inhibitors of angiogenesis (e.g. green tea, oranges, strawberries, lemons, red wine, ginseng, garlic, tomato, olive oil etc) there haven't been yet convincing clinical trials regarding their anti-obesity effect. Currently, the AngioLab Inc. is conducting phase II trials for the use of the **anti-angiogenic/anti-MMP drug, ALS-L1023** , in the treatment of obesity (183).

Sirtuin 1 (SIRT1) Activators

Sirtuin 1 (SIRT1) is a member of the sirtuin family of proteins that consists of seven members in mammals (SirT1-T7). Sirtuins have gained considerable attention due to their impact as physiological targets for treating diseases associated with aging. They contribute to cellular regulation interacting with metabolic pathways and may serve as entry points for drugs. SIRT1 in particular has gained popularity as it has been linked with the French Paradox and the calorie restriction-mediated longevity and delayed incidence of several diseases associated with aging, such as cancer, atherosclerosis, and diabetes; the above calorie restriction-induced modulations have been well established in organisms ranging from yeast to mammals (184). White adipose tissue (WAT) seems to be a primary tissue in longevity, as mice engineered to have reduced levels of WAT live longer (185). Supporting to this is the finding that food withdrawal is followed by SIRT1 binding to and repression of genes controlled by the fat regulator PPAR-γ (peroxisome proliferator-activated receptor-γ) including genes mediating fat storage which in turn activates fat mobilization, lipolysis and reduces WAT mass (186). In addition to PPAR-γ, SIRT1 also interacts with PGC-1α, inducing the expression of mitochondrial genes involved in oxidative metabolism and fatty acid oxidation while it also enhances leptin sensitivity by repressing PTP1B. The weight restricting effects of SIRT1 were further supported by experiments with **resveratrol** (RSV), a potent allosteric SIRT1 activator, which was shown to protect mice from diet-induced obesity (187). Furthermore, mice treated with **SRT 1720** , a potent, selective synthetic activator of SIRT1, were resistant to diet-induced obesity due to enhanced oxidative metabolism in skeletal muscle, liver, and brown adipose tissue indicating the positive metabolic consequences of specific SIRT1 activation (188). Currently, several pharmaceutical companies investigate specific **SIRT1 activators** in phase I and phase II trials for the treatment of type II diabetes and obesity (189) to define their utility in the treatment of obesity and metabolic diseases.

COMBINATION APPROACHES TO OBESITY TREATMENT – EYE ON THE FUTURE

As long as the prevalence of obesity rises, the need for better tolerated and more efficacious pharmacotherapies will increase. Recent advances in our knowledge about the energy homeostasis regulation at molecular level, allow novel anti-obesity drugs to be designed targeting specific molecules crucial for the energy balance modulation. There are drugs in development that induce satiety signaling, other that repress hunger or modulate nutrient

absorption and other that affect metabolism and lipogenesis in a way that energy expenditure exceeds energy intake. Experience however from multiple agents estimated has shown that a medication that targets only one mechanism produces weight loss of 5%–10%. This amount of weight loss may be enough to improve the metabolic profile and ameliorate cardiovascular risk factors, is however far away from what obese subjects would expect cosmetically and which usually means weight reduction of ~20%–25%, similar to what the bariatric surgeries offer. We thought we had promising reports coming from well designed studies addressing the weight loss efficacy of combined treatments with anti-obesity drugs targeting different appetite regulation molecules suggesting synergistic or even additive effect of the combined treatments on weight reduction but also maintenance of the weight loss in the long-term. The last few months however, FDA rejected three suggested antiobesity drugs Qnexa. Lorcaserine and Contrave due to concerns on their safety profile; among the rejected drugs were two that combined agents targeting different regulators of appetite/feeding system, wiping out hopes for a new medication to fight obesity in the immediate future. However, data from bariatric surgery weight loss and subsequent eating behavior modulations suggest that the mechanisms of long-term weight loss following bariatric surgery are insufficient to account for the resulting bodyweight loss alone (190-192) and that alteration of previously described gut hormones and neuroendocrine signaling may be actively involved in postoperative changes in eating behavior and appetite (193,194). Therefore, pharmacological intervention for the management of obesity in the future will be most likely a combination of the above neuropeptides in a way that mimics the changes underlying the surgically induced weight loss. The way however till we get the ‘bariatric pharmacotherapy’ is still long, with the most optimists suggesting promising successful deal with potent pharmacotherapy of obesity over the next decade. Table 1 and 2 summarizes current anti-obesity agents and combined drugs in progress.

Table 1. Anti-obesity drugs in progress

Drug	Drug description	Company
Metreleptin	leptin analogue	Takeda Pharmaceutical Company Limited
Pramlintide	Amylin anallog	Amylin Pharmaceuticals, Inc.
PTP1B blocker	Leptin sensitizer	
PBA	Leptin sensitizer	
TUDCA	Leptin sensitizer	
BMS-830216	MCH antagonist	Bristol-Myers Squibb
Lorcaserin (APD356)*	5-HT2C receptor agonists	Arena Pharmaceuticals
TTT-435	AgRP inhibitor	TransTech Pharma
Bupropion	dopamine and norepinephrine -reuptake inhibitor	
Zonisamide	serotonin-receptor agonist and dopamine-reuptake inhibitor	
Topiramate	GABA inhibitor	
S-2367	Y5-receptor antagonist	Shionogi USA, Inc
Obinepitide	Y2/Y4-receptor agonist	7TM Pharma
TM30339	Y4-receptor agonist	7TM Pharma
GI181771X	CCK1R agonist	GSK
Lixisenatide	GLP1R agonist	Zealand Pharma

CJC-1134-PC	GLP1R agonist	ConjuChem Biotechnologies Inc.
Albiglutide	GLP1R agonist	GSK
Taspoglutide	GLP1R agonist	Roche
LY2189265	GLP1R agonist	Eli Lilly
Liraglutide	GLP1R agonist	NovoNordisk
PYY3–36	Y2R agonist	
Oxyntomodulin	GLP1R agonist	
NOX-B11	Ghrelin vaccine	Pfizer
GOAT inhibitors	Ghrelin inhibitors	
ATL-962 (Cetilistat)		Alizyme
AOD-9604	GH lipolytic domain analogue	Phosphagenics
T-BVT	white adipose tissue selective 11β-HSD1 inhibitor	
SRT1720	SIRT1 activators	

* rejected by the FDA

Table 2. Combined treatments in development for the treatment of obesity	
Drug	**Study phase**
pramlintide/metreleptin	II
Bupropion/naltrexone (Contrave)*	rejected by the FDA
Bupropion / zonisamide (Empatic)	IIb
Topiramate/phentermine (Qnexa)*	rejected by the FDA
PYY3–36 /GLP-1 combination	II
PYY3–36/OXM	II
obinepitide (PP/PYY3-36)	II
amylin/PYY3–36	II
pramlintide/phentermine	II
pramlintide/sibutramine	II
β3-agonist LY-377604/ sibutramine	II
ALS-L1023	II

References

1. FDA: Guidance for industry developing products for weight management. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm071612.pdf .

2. Ahima RS, Flier JS. Leptin. Annu Rev Physiol. 2000;62:413-37

3. Ahima RS, Flier JS. Leptin. Annu Rev Physiol. 2000;62:413-37

4. Morton GJ , Blevins JE , Williams DL , Niswender KD , Gelling RW , Rhodes CJ , Baskin DG , Schwartz MW . Leptin action in the forebrain regulates the hindbrain response to satiety

signals. J Clin Invest. 2005 Mar;115(3):703-10.

5. Morton GJ, Matsen M, Figlewicz D, Schwartz MW. Leptin signaling in the ventral tegmental area enhances the satiety response to cholecystokinin. Diabetes. 2007;56:A403.

6. Montague CT , Farooqi IS , Whitehead JP , Soos MA , Rau H , Wareham NJ , Sewter CP , Digby JE , Mohammed SN , Hurst JA , Cheetham CH , Earley AR , Barnett AH , Prins JB , O'Rahilly S . Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature. 1997 Jun 26;387(6636):903-8

7. Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. J Clin Invest. 2005;115(12):3579-86

8. Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, et al. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. J Clin Invest. 2005;115(12):3579-86

9. Rosenbaum M, Murphy EM, Heymsfield SB, Matthews DE, Leibel RL. Low dose leptin administration reverses effects of sustained weight-reduction on energy expenditure and circulating concentrations of thyroid hormones. J Clin Endocrinol Metab. 2002;87:2391–2394.

10. Kissileff HR, Thornton JC, Torres M, et al. Maintenance of reduced body weight in humans is associated with leptin-reversible changes in appetite-related ratings [abstract]; Presented at the Society for Neuroscience Annual Meeting; San Diego, California. 3–7 November 2007.

11. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA. 1999;282(16):1568-75

12. Elchebly M, Payette P, Michaliszyn E, Cromlish W, Collins S, Loy AL, et al. Increased insulin sensitivity and obesity resistance in mice lacking the protein tyrosine phosphatase-1B gene. Science. 1999;283(5407):1544-8.

13. Picardi PK, Calegari VC, Prada PO, Moraes JC, Araújo E, Marcondes MC, et al. Reduction of hypothalamic protein tyrosine phosphatase improves insulin and leptin resistance in diet-induced obese rats. Endocrinology. 2008;149(8):3870-80.

14. Ozcan L, Ergin AS, Lu A, Chung J, Sarkar S, Nie D, Myers MG, Jr, Ozcan U. Endoplasmic reticulum stress plays a central role in development of leptin resistance. Cell Metabolism. 2009;9:35–51

15. Roth JD, Roland BL, Cole RL, Trevaskis JL, Weyer C, Koda JE, Anderson CM, Parkes DG, Baron AD. Leptin responsiveness restored by amylin agonism in diet-induced obesity: Evidence from nonclinical and clinical studies. Proc. Natl. Acad. Sci. U. S. A. 2008;105:7257–7262

16. Amylin Pharmaceuticals, Inc. Available from: http://www.takeda.com/press/article_35851.html

17. Takeda Pharmaceutical Company Limited. Available from: http://www.takeda.com/press/article_42791.html

18. Fehm HL, Smolnik R, Kern W, McGregor GP, Bickel U, Born J. The melanocortin melanocyte-stimulating hormone adren-ocorticotropin(4-10) decreases body fat in humans. J Clin Endocrinol Metab. 2001;86(3):1144-8

19. Pissios P, Bradley RL, Maratos-Flier E. Expanding the scales: the multiple roles of MCH in regulating energy balance and other biological functions. Endocr Rev. 2006;27(6):606-20

20. Bristol-Myers Squibb metabolics clinical trial registry. Available from: http://ctr.bms.com/OneBmsCtd/InitTrialAction.do?linkname=Metabolics&type=pharma&sortby=default .

21. Launay JM, Herve P, Peoc'h K, Tournois C, Callebert J, Nebigil CG, Etienne N, Drouet L, Humbert M, Simonneau G and Maroteaux L (2002) Function of the serotonin 5-hydroxytryptamine 2B receptor in pulmonary hypertension. Nature Medicine 8:1129-1135.

22. Fitzgerald LW, Burn TC, Brown BS, Patterson JP, Corjay MH, Valentine PA, Sun J-H, Link JR, Abbaszade I, Hollis JM, Largent BL, Hartig PR, Hollis GF, Meunier PC, Robichaud AJ and Robertson DW (2000) ACCELERATED COMMUNICATION: Possible Role of Valvular Serotonin 5-HT2B Receptors in the Cardiopathy Associated with Fenfluramine. Mol Pharmacol 57:75-81.

23. Dunlop J, Sabb A and Mazandarani H (2005) WAY-163909 [(7bR, 10aR)-1,2,3,4,8,9,10, 10a-octahydro-7bH-cyclopenta-[b][1,4]diazepino[6,7,1 hi]indole], a novel 5- hydroxytryptamine 2C receptor-selective agonist with anorectic activity. J. Pharmacol. Exp. Ther. 313:862-869.

24. Siuciak JA, Chapin DS, McCarthy SA, Guanowsky V, Brown J, Chiang P, Marala R, Patterson T, Seymour PA, Swick A and Iredale PA (2007) CP-809,101, a JPET #179572 25 selective 5-HT2C agonist, shows activity in animal models of antipsychotic activity. Neuropharmacology 52:279-290.

25. Ramamoorthy P, Beyer C, Brennan J, Dunlop J, Gove S, Grauer S, Harrison BL, Lin Q, Malberg J, Marquis K, Mazandarani H, Piesla M, Pulicicchio C, RosenzweigLipson S, Sabb A-M, Schechter L, Stack G and J Z (2006) Discovery of SCA-136, a novel 5-HT2C agonist, for the treatment of schizophrenia. . 231st American Chemical Society National Meeting:MEDI-021.

26. Thomsen WJ, Grottick AJ, Menzaghi F, Reyes-Saldana H, Espitia S, Yuskin D, Whelan K, Martin M, Morgan M, Chen W, Al-Shamma H, Smith B, Chalmers D and Behan D (2008) Lorcaserin, a Novel Selective Human 5-Hydroxytryptamine2C Agonist: in Vitro and in Vivo Pharmacological Characterization. J Pharmacol Exp Ther 325:577-587.

27. Arena Pharmaceuticals Announces Positive Phase 2b Clinical Trial Results of Novel Anti-

Obesity Compound. Available from:

http:// www.invest.arenapharm.com/releasedetail.cfm?ReleaseID=320421

28. Complete Response Letter for Lorcaserin NDA. FDA Issues Complete Response Letter for Lorcaserin New Drug Application. Available from: http://www.drugs.com/nda/lorcaserin_101025.html .

29. TransTech Pharma obesity product pipeline. Available from: http://www.ttpharma.com/TherapeuticAreas/MetabolicDiseases/Obesity/TTP435/tabid/126/Default.aspx .

30. Kirkham TC, Tucci SA. Endocannabinoids in appetite control and the treatment of obesity. CNS Neurol Disord Drug Targets. 2006;5(3):272-92.

31. Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a metaanalysis of randomised trials. Lancet.2007;370(9600):1706-13.

32. Zarrindast MR , Hosseini-Nia T . Anorectic and behavioural effects of bupropion. Gen Pharmacol. 1988;19(2):201-4.

33. Billes SK , Cowley MA . Inhibition of dopamine and norepinephrine reuptake produces additive effects on energy balance in lean and obese mice. Neuropsychopharmacology. 2007 Apr;32(4):822-34.

34. Gadde KM , Yonish GM , Wagner HR 2nd , Foust MS , Allison DB . Atomoxetine for weight reduction in obese women: a preliminary randomised controlled trial. Int J Obes (Lond). 2006 Jul;30(7):1138-42.

35. Greenway FL, Whitehouse MJ, Guttadauria M, Anderson JW, Atkinson RL, Fujioka K, Gadde KM, Gupta AK, O'Neil P, Schumacher D, Smith D, Dunayevich E, Tollefson GD, Weber E, Cowley MA 2009 Rational design of a combination medication for the treatment of obesity. Obesity (Silver Spring) 17:30–39

36. Anderson JW, Greenway FL, Fujioka K, Gadde KM, McKenney J, O'Neil PM. Bupropion SR enhances weight loss: a 48-week double-blind, placebo- controlled trial. Obes Res. 2002;10(7):633-41.

37. Jain AK , Kaplan RA , Gadde KM , Wadden TA , Allison DB , Brewer ER , Leadbetter RA , Richard N , Haight B , Jamerson BD , Buaron KS , Metz A . Bupropion SR vs. placebo for weight loss in obese patients with depressive symptoms. Obes Res. 2002 Oct;10(10):1049-56.

38. Gadde KM , Parker CB , Maner LG , Wagner HR 2nd , Logue EJ , Drezner MK , Krishnan KR . Bupropion for weight loss: an investigation of efficacy and tolerability in overweight and obese women. Obes Res. 2001 Sep;9(9):544-51.

39. Cowley MA, Smart JL, Rubinstein M, Cerdán MG, Diano S, Horvath TL, Cone RD, Low MJ 2001 Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411:480–484

40. Yeomans MR, Gray RW 2002 Opioid peptides and the control of human ingestive behaviour. Neurosci Biobehav Rev 26:713–728

41. Atkinson RL, Berke LK, Drake CR, Bibbs ML, Williams FL, Kaiser DL 1985 Effects of long-term therapy with naltrexone on body weight in obesity. Clin Pharmacol Ther 38:419–422

42. Mitchell JE, Morley JE, Levine AS, Hatsukami D, Gannon M, Pfohl D 1987 High-dose naltrexone therapy and dietary counseling for obesity. Biol Psychiatry 22:35–42

43. Malcolm R, O'Neil PM, Sexauer JD, Riddle FE, Currey HS, Counts C 1985 A controlled trial of naltrexone in obese humans. Int J Obes 9:347–353

44. Greenway FL , Fujioka K , Plodkowski RA , Mudaliar S , Guttadauria M , Erickson J , Kim DD , Dunayevich E ; COR-I Study Group . Effect of naltrexone plus bupropion on weight loss in overweight and obese adults (COR-I): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet. 2010 Aug 21;376(9741):595-605.

45. Wadden TA , Foreyt JP , Foster GD , Hill JO , Klein S , O'Neil PM , Perri MG , Pi-Sunyer FX , Rock CL , Erickson JS , Maier HN , Kim DD , Dunayevich E . Weight loss with naltrexone SR/bupropion SR combination therapy as an adjunct to behavior modification: the COR-BMOD trial. Obesity (Silver Spring). 2011 Jan;19(1):110-20.

46. Okada M, Kaneko S, Hirano T, et al. Effects of zonisamide on dopaminergic system. Epilepsy Res. 1995;22:193-205.

47. Okada M, Hirano T, Kawata Y, et al. Biphasic effects of zonisamide on serotonergic system in rat hippocampus. Epilepsy Res. 1999;34:187-197.

48. Gadde KM , Franciscy DM , Wagner HR 2nd , Krishnan KR . Zonisamide for weight loss in obese adults: a randomized controlled trial. JAMA. 2003 Apr 9;289(14):1820-5.

49. Lim J , Ko YH , Joe SH , Han C , Lee MS , Yang J . Zonisamide produces weight loss in psychotropic drug-treated psychiatric outpatients. Prog Neuropsychopharmacol Biol Psychiatry. 2011 Dec 1;35(8):1918-21.

50. McElroy SL , Kotwal R , Hudson JI , Nelson EB , Keck PE . Zonisamide in the treatment of binge-eating disorder: an open-label, prospective trial. J Clin Psychiatry. 2004 Jan;65(1):50-6.

51. McElroy SL , Kotwal R , Guerdjikova AI , Welge JA , Nelson EB , Lake KA , D'Alessio DA , Keck PE , Hudson JI . Zonisamide in the treatment of binge eating disorder with obesity: a randomized controlled trial. J Clin Psychiatry. 2006 Dec;67(12):1897-906.

52. Orexigen Therapeutics, Inc. Press Release. Orexigen® Therapeutics Phase 2b Trial for Empatic™ Meets Primary Efficacy Endpoint Demonstrating Significantly Greater Weight Loss Versus Comparators in Obese Patients. Available from: http://ir.orexigen.Com/Phoenix.Zhtml?C=207034&P=Irol-Newsarticle&Id=1336796&Highlight

53. Gadde KM , Yonish GM , Foust MS , Wagner HR . Combination therapy of zonisamide and bupropion for weight reduction in obese women: a preliminary, randomized, open-label study. J Clin Psychiatry. 2007 Aug;68(8):1226-9.

54. Roose SP, Dalack GW, Glassman AH, Woodring S, Walsh BT, Giardina EG. Cardiovascular effects of bupropion in depressed patients with heart disease. Am. J. Psychiatry 148(4), 512–516 (1991).

55. ZYBAN® (bupropion hydrochloride) sustained-release tablets. Available from: www.biopsychiatry.com/bupropion/bupropion-zyban.pdf

56. Mula M, Sander JW. Negative effects of antiepileptic drugs on mood in patients with epilepsy. *Drug Saf.* 30(7), 555–567 (2007).

57. Ohtahara S, Yamatogi Y. Safety of zonisamide therapy: prospective follow-up survey. *Seizure* 13(Suppl. 1), S50–S55; discussion S56 (2004).

58. Topiramate (Topamax®) tablets. Physicians' Desk Reference. Medical Economics Co., Inc: Montvale, NJ; 2002. pp 2590–2595.

59. Rosenfeld WE. Topiramate: a review of preclinical, pharmacokinetic, and clinical data. Clin Ther. 1997;19(6):1294-308.

60. Bray GA, Hollander P, Klein S, Kushner R, Levy B, Fitchet M, Perry BH, for the US Topiramate Research Group. A 6-month randomized, placebo-controlled dose-ranging trial of topiramate for weight loss in obesity. Obes Res 2003; 11: 722–733.

61. Wilding J , Van Gaal L , Rissanen A , Vercruysse F , Fitchet M ; OBES-002 Study Group . A randomized double-blind placebo-controlled study of the long-term efficacy and safety of topiramate in the treatment of obese subjects. Int J Obes Relat Metab Disord. 2004 Nov;28(11):1399-410.

62. McElroy SL , Arnold LM , Shapira NA , Keck PE Jr , Rosenthal NR , Karim MR , Kamin M , Hudson JI . Topiramate in the treatment of binge eating disorder associated with obesity: a randomized, placebo-controlled trial. Am J Psychiatry. 2003 Feb;160(2):255-61.

63. Hoopes SP , Reimherr FW , Hedges DW , Rosenthal NR , Kamin M , Karim R , Capece JA , Karvois D . Treatment of bulimia nervosa with topiramate in a randomized, double-blind, placebo-controlled trial, part 1: improvement in binge and purge measures. J Clin Psychiatry. 2003 Nov;64(11):1335-41.

64. Gadde KM, Allison DB, Ryan DH, Peterson CA, Troupin B, Schwiers ML, Day WW. Effects of low-dose, controlled-release, phentermine plus topiramate combination on weight and associated comorbidities in overweight and obese adults (CONQUER): a randomised, placebo-controlled, phase 3 trial. Lancet. 2011 Apr 16;377(9774):1341-52. Epub 2011 Apr 8. Erratum in: Lancet. 2011 Apr 30;377(9776):1494.

65. VIVUS: Qnexa meets primary endpoint by demonstrating superior weight loss over components and placebo in the 28-week equate study (OB-301). Available from: http://ir.vivus.com/releasedetail.cfm?releaseid=353965

66. VIVUS Inc. Press releases (2010). Available from: http://ir.vivus.com/releases.cfm?year=&releasestype=&pagenum=2

67. Vivus. FDA issues complete response letter to VIVUS regarding new drug application for QNEXA [press release]. October 28, 2010.

68. Broberger C, Landry M, Wong H, Walsh JN, Hökfelt T. Subtypes Y1 and Y2 of the neuropeptide Y receptor are respectively expressed in pro-opiomelanocortin-and neuropeptide-Y-containing neurons of the rat hypothalamic arcuate nucleus. Neuroendocrinology. 1997;66(6):393-408.

69. Erondu N, Gantz I, Musser B, Suryawanshi S, Mallick M, Addy C, et al. Neuropeptide Y5 receptor antagonism does not induce clinically meaningful weight loss in overweight and obese adults. Cell Metab. 2006;4(4):275-82.

70. Sargent BJ , Moore NA . New central targets for the treatment of obesity. Br J Clin Pharmacol. 2009 Dec;68(6):852-60.

71. Gibbs, J., Young, R.C., Smith, G.P. 1973. Cholycystokinin elicits satiety in rats with open gastric fistulas. Nature. 245:323-325.

72. Moran, T.H., Katz, L.F., Plata-Salaman, C.R., Schwartz, G.J. 1998. Disordered food intake and obesity in rats lacking cholecystokinin A receptors. Am. J. Physiol. 274:R618-R625.

73. Kopin, A.S., et al. 1999. The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. J. Clin. Invest. 103:383-391.

74. Moran, T.H., Kinzig, K.P. 2004. Gastrointestinal satiety signals. II. Cholecystokinin. Am. J. Physiol. Gastrointest. Liver Physiol. 286:G183-G188.

75. Zittel, T.T., et al. 1999. C-fos protein expression in the nucleus of the solitary tract correlates with cholecystokinin dose injected and food intake in rats. Brain Res. 846:1-11.

76. Smith, G.P., Jerome, C., Cushin, B.J., Eterno, R., Simansky, K.J. 1981. Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. Science. 213:1036-1037.

77. Moran, T.H., Baldessarini, A.R., Salorio, C.F., Lowery, T., Schwartz, G.J. 1997. Vagal afferent and efferent contributions to the inhibition of food intake by cholecystokinin. Am. J. Physiol. 272:R1245-R1251.

78. Joyner, K., Smith, G.P., Gibbs, J. 1993. Abdominal vagotomy decreases the satiating potency of CCK-8 in sham and real feeding. Am. J. Physiol. 264:R912-R916.

79. Schwartz, G.J., McHugh, P.R., Moran, T.H. 1993. Gastric loads and cholecystokinin synergistically stimulate rat gastric vagal afferents. Am. J. Physiol. 265:R872-R876.

80. Moran, T.H., McHugh, P.R. 1982. Cholecystokinin suppresses food intake by inhibiting gastric emptying. Am. J. Physiol. 242:R491-R497.

81. Kissileff, H.R., Carretta, J.C., Geliebter, A., Pi-Sunyer, F.X. 2003. Cholecystokinin and stomach distension combine to reduce food intake in humans. Am. J. Physiol. Regul. Integr. Comp. Physiol.285:R992-R998.

82. M. O. Weickert, M. Mohlig, J. Spranger, C. Schofl, C. V. Loeffelholz, R. L. Riepl, B. Otto, and A. F. H. Pfeiffer. Effects of Euglycemic Hyperinsulinemia and Lipid Infusion on Circulating Cholecystokinin. J Clin Endocrinol Metab. 2008,93(6):2328–2333

83. Kissileff, H.R., Pi-Sunyer, F.X., Thornton, J., Smith, G.P. 1981. C-terminal octapeptide of cholecystokinin decreases food intake in man. Am. J. Clin. Nutr. 34:154-160.

84. Muurahainen, N., Kissileff, H.R., Derogatis, A.J., Pi-Sunyer, F.X. 1988. Effects of cholecystokinin-octapeptide (CCK-8) on food intake and gastric emptying in man. Physiol. Behav. 44:645-649.

85. Crawley, J.N., Beinfeld, M.C. 1983. Rapid development of tolerance to the behavioural actions of cholecystokinin. Nature. 302:703-706.

86. Pi-Sunyer X, Kissileff HR, Thornton J, Smith GP. C-terminal octapeptide of cholecystokinin decreases food intake in obese men. Physiol Behav. 1982 Oct;29(4):627-30.

87. West, D.B., Fey, D., Woods, S.C. 1984. Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. Am. J. Physiol. 246:R776-R787.

88. Emma Janet Castillo, Silvia Delgado-Aros , Michael Camilleri,Duane Burton, Debra Stephens, Robin O'Connor-Semmes, Ann Walker, Anne Shachoy-Clark, and Alan R. Zinsmeister. Effect of oral CCK-1 agonist GI181771X on fasting and postprandial gastric functions in healthy volunteers. AJP – GI August 2004 vol. 287 no. 2 G363-G369

89. Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., Baskin, D.G. 2000. Central nervous system control of food intake. Nature. 404:661-671.

90. Morton, G.J., Cummings, D.E., Baskin, D.G., Barsh, G.S., Schwartz, M.W. 2006. Central

nervous system control of food intake and body weight. Nature. 443:289-295.

91. Lee JY, Ye J, Gao Z, Youn HS, Lee WH, Zhao L, et al. Reciprocal modulation of Toll-like receptor-4 signaling pathways involving MyD88 and phosphatidylinositol 3-kinase/AKT by saturated and polyunsaturated fatty acids. J Biol Chem. 2003;278(39):37041-51

92. Verdich, C., et al. 2001. A meta-analysis of the effect of glucagon-like peptide-1 (7-36) amide on ad libitum energy intake in humans. J. Clin. Endocrinol. Metab. 86:4382-4389.

93. Gutzwiller, J.P., et al. 1999. Glucagon-like peptide-1: a potent regulator of food intake in humans. Gut. 44:81-86.

94. Naslund, E., et al. 1999. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. Int. J. Obes. Relat. Metab. Disord.23:304-311.

95. Toft-Nielsen, M.B., Madsbad, S., Holst, J.J. 1999. Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. Diabetes Care. 22:1137-1143.

96. Zander, M., Madsbad, S., Madsen, J.L., Holst, J.J. 2002. Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. Lancet. 359:824-830.

97. Henry, R., et al. 2006. Exenatide maintained glycemic control with associated weight reduction over 2 years in patients with type 2 diabetes [abstract]. In66th Scientific Sessions of the American Diabetes Association. June 9–13, 2006. Washington, DC, USA. 485-P.

98. Abbott, C.R., et al. 2005. The inhibitory effects of peripheral administration of peptide YY(3-36) and glucagon-like peptide-1 on food intake are attenuated by ablation of the vagal-brainstem-hypothalamic pathway. Brain Res. 1044:127-131.

99. Talsania, T., Anini, Y., Siu, S., Drucker, D.J., Brubaker, P.L. 2005. Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. Endocrinology. 146:3748-3756.

100. Baggio, L.L., Huang, Q., Brown, T.J., Drucker, D.J. 2004. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. Gastroenterology. 127:546-558.

101. Drucker, D.J. 2006. The biology of incretin hormones. Cell Metab. 3:153-165.

102. Astrup A, Rössner S, Van Gaal L, et al. Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. Lancet 2009; 374:1606-1616.

103. Zealand Pharma product pipeline. Available from: www.zp.dk/%7BD566D263-F216–4A37-A38F-8C33A4ECC4E0%7D

104. Barnett AH . Lixisenatide: evidence for its potential use in the treatment of type 2 diabetes. Core Evid. 2011;6:67-79.

105. Pinelli NR , Hurren KM . Efficacy and safety of long-acting glucagon-like peptide-1 receptor agonists compared with exenatide twice daily and sitagliptin in type 2 diabetes mellitus: a systematic review and meta-analysis. Ann Pharmacother. 2011 Jul;45(7-8):850-60.

106. Madsbad S , Kielgast U , Asmar M , Deacon CF , Torekov SS , Holst JJ . An overview of once-weekly glucagon-like peptide-1 receptor agonists-available efficacy and safety data and perspectives for the future. Diabetes Obes Metab. 2011 May;13(5):394-407. doi: 10.1111/j.1463-1326.2011.01357.x.

107. Robert E Steinert , Birk Poller , M Cristina Castelli , Juergen Drewe , and Christoph Beglinger . Oral administration of glucagon-like peptide 1 or peptide YY 3-36 affects food intake in healthy male subjects. Am J Clin Nutr October 2010 vol. 92 no. 4 810-817

108. Beglinger C, Poller B, Arbit E, et al. Pharmacokinetics and pharmacodynamic effects of oral GLP-1 and PYY3-36: a proof-of-concept study in healthy subjects. Clin Pharmacol Ther 2008;84:468–74.

109. Steinert RE, Poller B, Castelli MC, et al. Orally administered glucagon-like peptide-1 affects glucose homeostasis following an oral glucose tolerance test in healthy male subjects. Clin Pharmacol Ther 2009;86(6):644–50.

110. Pironi, L., et al. 1993. Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY. Gastroenterology.105:733-739.

111. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, et al. Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med. 2003;349(10):941-8.

112. Degen L , Oesch S , Casanova M , Graf S , Ketterer S , Drewe J , Beglinger C . Effect of peptide YY3-36 on food intake in humans. Gastroenterology. 2005 Nov;129(5):1430-6.

113. Field BC , Wren AM , Peters V , Baynes KC , Martin NM , Patterson M , Alsaraf S , Amber V , Wynne K , Ghatei MA , Bloom SR . PYY3-36 and oxyntomodulin can be additive in their effect on food intake in overweight and obesehumans. Diabetes. 2010 Jul;59(7):1635-9.

114. Gantz I, Erondu N, Mallick M, Musser B, Krishna R, Tanaka WK, et al. Efficacy and safety of intranasal peptide YY3-36 for weight reduction in obese adults. J Clin Endocrinol Metab. 2007;92(5):1754-7.

115. Talsania, T., Anini, Y., Siu, S., Drucker, D.J., Brubaker, P.L. 2005. Peripheral exendin-4 and peptide YY(3-36) synergistically reduce food intake through different mechanisms in mice. Endocrinology. 146:3748-3756.

116. Scott, V., Kimura, N., Stark, J.A., Luckman, S.M. 2005. Intravenous peptide YY3-36 and

Y2 receptor antagonism in the rat: effects on feeding behaviour. J. Neuroendocrinol. 17:452-457.

117. Halatchev, I.G., Ellacott, K.L., Fan, W., Cone, R.D. 2004. Peptide YY3-36 inhibits food intake in mice through a melanocortin-4 receptor-independent mechanism. Endocrinology. 145:2585-2590.

118. Adams, S.H., Won, W.B., Schonhoff, S.E., Leiter, A.B., Paterniti, J.R. 2004. Effects of peptide YY[3-36] on short-term food intake in mice are not affected by prevailing plasma ghrelin levels. Endocrinology. 145:4967-4975.

119. Challis, B.G., et al. 2004. Mice lacking POMC are sensitive to high-fat feeding but respond normally to the acute anorectic effects of peptide-YY(3-36). Proc. Natl. Acad. Sci. U. S. A. 101:4695-4700.

120. Pittner, R.A., et al. 2004. Effects of PYY[3-36] in rodent models of diabetes and obesity. Int. J. Obes. Relat. Metab. Disord. 28:963-971.

121. Chelikani, P.K., Haver, A.C., Reeve, J.R., Keire, D.A., Reidelberger, R.D. 2006. Daily, intermittent intravenous infusion of peptide YY(3-36) reduces daily food intake and adiposity in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 290:R298-R305.

122. Moran, T.H., et al. 2005. Peptide YY(3-36) inhibits gastric emptying and produces acute reductions in food intake in rhesus monkeys. Am. J. Physiol. Regul. Integr. Comp. Physiol. 288:R384-R388.

123. Batterham, R.L., et al. 2002. Gut hormone PYY(3-36) physiologically inhibits food intake. Nature. 418:650-654.

124. 7TM Pharma. 7TM Pharma’s new first-in-class anti-obesity drug completes Phase I/II clinical studies. Press release [online] 2006.

125. Dakin, C.L., et al. 2001. Oxyntomodulin inhibits food intake in the rat. Endocrinology. 142:4244-4250.

126. Dakin, C.L., et al. 2004. Peripheral oxyntomodulin reduces food intake and body weight gain in rats. Endocrinology. 145:2687-2695.

127. Baggio, L.L., Huang, Q., Brown, T.J., Drucker, D.J. 2004. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. Gastroenterology. 127:546-558.

128. Yamamoto, H., et al. 2002. Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. J. Clin. Invest. 110:43-52.

129. Cohen, M.A., et al. 2003. Oxyntomodulin suppresses appetite and reduces food intake in

humans. J. Clin. Endocrinol. Metab. 88:4696-4701.

130. Wynne K, Park AJ, Small CJ, Patterson M, Ellis SM, Murphy KG, et al. Subcutaneous oxyntomodulin reduces body weight in overweight and obese subjects: a double-blind, randomized, controlled trial. Diabetes. 2005;54(8):2390-5

131. Wynne, K., et al. 2006. Oxyntomodulin increases energy expenditure in addition to decreasing energy intake in overweight and obese humans: a randomised controlled trial. Int. J. Obes. (Lond.). 30:1729-1736.

132. Santoprete A , Capitò E , Carrington PE , Pocai A , Finotto M , Langella A , Ingallinella P , Zytko K , Bufali S , Cianetti S , Veneziano M , Bonelli F , Zhu L , Monteagudo E , Marsh DJ , Sinharoy R , Bianchi E , Pessi A . DPP-IV-resistant, long-acting oxyntomodulin derivatives. J Pept Sci. 2011 Apr;17(4):270-80.

133. Kerr BD , Flatt PR , Gault VA . (D-Ser2)Oxm[mPEG-PAL]: a novel chemically modified analogue of oxyntomodulin with antihyperglycaemic, insulinotropic and anorexigenic actions. Biochem Pharmacol. 2010 Dec 1;80(11):1727-35.

134. Cummings, D.E., Foster-Schubert, K.E., Overduin, J. 2005. Ghrelin and energy balance: focus on current controversies. Curr. Drug Targets.6:153-169.

135. Date Y , Murakami N , Toshinai K , Matsukura S , Niijima A , Matsuo H , Kangawa K , Nakazato M . The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. Gastroenterology. 2002 Oct;123(4):1120-8.

136. Wren, A.M., et al. 2001. Ghrelin enhances appetite and increases food intake in humans. J. Clin. Endocrinol. Metab. 86:5992-5995.

137. Zorrilla EP , Iwasaki S , Moss JA , Chang J , Otsuji J , Inoue K , Meijler MM , Janda KD . Vaccination against weight gain. Proc Natl Acad Sci U S A. 2006 Aug 29;103(35):13226-31.

138. Moran TH , Dailey MJ . Minireview: Gut peptides: targets for antiobesity drug development? Endocrinology. 2009 Jun;150(6):2526-30.

139. Xin Z , Serby MD , Zhao H , Kosogof C , Szczepankiewicz BG , Liu M , Liu B , Hutchins CW , Sarris KA , Hoff ED , Falls HD , Lin CW , Ogiela CA , Collins CA , Brune ME , Bush EN , Droz BA , Fey TA , Knourek-Segel VE , Shapiro R , Jacobson PB , Beno DW , Turner TM , Sham HL , Liu G . Discovery and pharmacological evaluation of growth hormone secretagogue receptor antagonists. J Med Chem. 2006 Jul 27;49(15):4459-69.

140. Yang J, Zhao T-J, Goldstein JL, Brown MS. Inhibition of ghrelin O-acyltransferase (GOAT) by octanoylated pentapeptides. Proceedings of the National Academy of Sciences.2008;105:10750–10755.

141. Okada, S., York, D.A., Bray, G.A., Mei, J., Erlanson-Albertsson, C. 1992. Differential inhibition of fat intake in two strains of rat by the peptide enterostatin. Am. J. Physiol. 262:R1111-R1116.

142. Kovacs, E.M., Lejeune, M.P., Westerterp-Plantenga, M.S. 2003. The effects of enterostatin intake on food intake and energy expenditure.Br. J. Nutr. 90:207-214.

143. Morton NM, Emilsson V, Liu YL, and Cawthorne MA. Leptin action in intestinal cells. J Biol Chem 273: 26194–26201, 1998.

144. Doi T, Liu M, Seeley RJ, Woods SC, and Tso P. Effect of leptin on intestinal apolipoprotein AIV in response to lipid feeding. Am J Physiol Regul Integr Comp Physiol 281: R753–R759, 2001.

145. Attia N, Touzani A, Lahrichi M, Balafrej A, Kabbaj O, and Girard-Globa A. Response of apolipoprotein AIV and lipoproteins to glycaemic control in young people with insulin-dependent diabetes mellitus. Diabet Med 14: 242–247, 1997.

146. Black DD and Ellinas H. Apolipoprotein synthesis in newborn piglet intestinal explants. Pediatr Res 32: 553–558, 1992.

147. Fujimoto K, Cardelli JA, and Tso P. Increased apolipoprotein A-IV in rat mesenteric lymph after lipid meal acts as a physiological signal for satiation. Am J Physiol Gastrointest Liver Physiol 262: G1002–G1006, 1992.

148. Fujimoto K, Machidori H, Iwakiri R, Yamamoto K, Fujisaki J, Sakata T, and Tso P. Effect of intravenous administration of apolipoprotein A-IV on patterns of feeding, drinking and ambulatory activity of rats. Brain Res 608: 233–237, 1993.

149. Katsuura, G., Asakawa, A., Inui, A. 2002. Roles of pancreatic polypeptide in regulation of food intake. Peptides. 23:323-329.

150. Asakawa A , Inui A , Ueno N , Fujimiya M , Fujino MA , Kasuga M . Mouse pancreatic polypeptide modulates food intake, while not influencing anxiety in mice. Peptides. 1999 Dec;20(12):1445-8.

151. Batterham RL , Le Roux CW , Cohen MA , Park AJ , Ellis SM , Patterson M , Frost GS , Ghatei MA , Bloom SR . Pancreatic polypeptide reduces appetite and food intake in humans. J Clin Endocrinol Metab. 2003 Aug;88(8):3989-92.

152. Jesudason DR , Monteiro MP , McGowan BM , Neary NM , Park AJ , Philippou E , Small CJ , Frost GS , Ghatei MA , Bloom SR . Low-dose pancreatic polypeptide inhibits food intake in man. Br J Nutr. 2007 Mar;97(3):426-9.

153. Neary NM , McGowan BM , Monteiro MP , Jesudason DR , Ghatei MA , Bloom SR . No evidence of an additive inhibitory feeding effect following PP and PYY 3-36 administration. Int J

Obes (Lond). 2008 Sep;32(9):1438-40.

154. Lutz, T.A., Althaus, J., Rossi, R., Scharrer, E. 1998. Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers. Am. J. Physiol. 274:R1777-R1782.

155. Roth JD, Anderson, C 2006 Antiobesity effects of the β-cell hormone amylin in diet-induced obese rats: effects on food intake, body weight, composition, energy expenditure, and gene expression. Endocrinology 147:5855–5864

156. Rushing, P.A., Hagan, M.M., Seeley, R.J., Lutz, T.A., Woods, S.C. 2000. Amylin: a novel action in the brain to reduce body weight.Endocrinology. 141:850-853.

157. Lutz, T.A., Geary, N., Szabady, M.M., Del Prete, E., Scharrer, E. 1995. Amylin decreases meal size in rats. Physiol. Behav. 58:1197-1202.

158. Young AA 1997 Amylin's physiology and its role in diabetes. Curr Opin Endocrinol Diab4:282–290

159. Young AA, Vine W, Gedulin BR, Pittner R, Janes S, Gaeta LSL, Percy A, Moore CX, Koda JE, Rink TJ, Beaumont K 1996 Preclinical pharmacology of pramlintide in the rat: comparisons with human and rat amylin. Drug Dev Res 37:231–248

160. Hollander, P., et al. 2004. Effect of pramlintide on weight in overweight and obese insulin-treated type 2 diabetes patients. Obes. Res.12:661-668.

161. Chapman I, Parker B, Doran S, Feinle-Bisset C, Wishart J, Strobel S, Wang Y, Burns C, Lush C, Weyer C, Horowitz M 2005 Effect of pramlintide on satiety and food intake in obese subjects and subjects with type 2 diabetes. Diabetologia 48:838–848

162. Hollander PA, Levy P, Fineman MS, Maggs DG, Shen LZ, Strobel SA, Weyer C, Kolterman OG 2003 Pramlintide as an adjunct to insulin therapy improves long-term glycemic and weight control in patients with type 2 diabetes: a 1-year randomized controlled trial. Diabetes Care 26:784–790

163. Aronne L, Fujioka K, Aroda V, Chen K, Halseth A, Kesty NC, et al. Progressive reduction in body weight after treatment with the amylin analog pramlintide in obese subjects: a phase 2, randomized, placebo-controlled, dose-escalation study. J Clin Endocrinol Metab. 2007;92(8):2977-83.

164. Roth JD , Roland BL , Cole RL , Trevaskis JL , Weyer C , Koda JE , Anderson CM , Parkes DG , Baron AD . Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc Natl Acad Sci U S A. 2008 May 20;105(20):7257-62.

165. Trevaskis JL , Coffey T , Cole R , Lei C , Wittmer C , Walsh B , Weyer C , Koda J , Baron AD , Parkes DG , Roth JD . Amylin-mediated restoration of leptin responsiveness in diet-

induced obesity: magnitude and mechanisms. Endocrinology. 2008 Nov;149(11):5679-87.

166. Roth JD , Coffey T , Jodka CM , Maier H , Athanacio JR , Mack CM , Weyer C , Parkes DG . Combination therapy with amylin and peptide YY[3-36] in obese rodents: anorexigenic synergy and weight loss additivity. Endocrinology. 2007 Dec;148(12):6054-61.

167. Aronne LJ , Halseth AE , Burns CM , Miller S , Shen LZ . Enhanced weight loss following coadministration of pramlintide with sibutramine or phentermine in a multicenter trial. Obesity (Silver Spring). 2010 Sep;18(9):1739-46.

168. Kopelman P , Bryson A , Hickling R , Rissanen A , Rossner S , Toubro S , Valensi P . Cetilistat (ATL-962), a novel lipase inhibitor: a 12-week randomized, placebo-controlled study of weight reduction in obese patients. Int J Obes (Lond). 2007 Mar;31(3):494-9.

169. Bryson A , de la Motte S , Dunk C . Reduction of dietary fat absorption by the novel gastrointestinal lipase inhibitor cetilistat in healthy volunteers. Br J Clin Pharmacol. 2009 Mar;67(3):309-15.

170. Kopelman P , Groot Gde H , Rissanen A , Rossner S , Toubro S , Palmer R , Hallam R , Bryson A , Hickling RI . Weight loss, HbA1c reduction, and tolerability of cetilistat in a randomized, placebo-controlled phase 2 trial in obese diabetics: comparison with orlistat (Xenical). Obesity (Silver Spring). 2010 Jan;18(1):108-15.

171. Scacchi, M., Pincelli, A. I., Cavagnini, F. (1999) Growth hormone in obesity.Int J Obes Relat Metab Disord. 23: 260–271.

172. Shadid S , Jensen MD . Effects of growth hormone administration in human obesity. Obes Res. 2003 Feb;11(2):170-5.

173. The Phase 2B trial results for Metabolic's drug, AOD9604, do not support the commercial viability of the drug as a treatment for obesity. Development of the drug for this condition is terminated. Available from: http://www.metabolic.com.au/files/POQ26IMG5C/ASX_OPTIONSStudyResults_21Feb2007.pdf .

174. Mathvink, R.J. et al. Discovery of a potent, orally bioavailable β3 adrenergic receptor agonist,(R)-N-[4-[2-[[2-Hydroxy-2-(3-pyridinyl)ethyl]amino]ethyl]phenyl]-4-[4-[4-(tri fluoromethyl)phenyl]thiazol-2-yl]benzenesulfonamide. Journal of Medicinal Chemistry 43, 3832-6 (2000).

175. Van Baak, M.A. et al. Acute effect of L-796568, a novel β3-adrenergic receptor agonist, on energy expenditure in obese men. Clinical Pharmacology and Therapeutics 71, 272-9 (2002).

176. Larsen, T.M. et al. Effect of a 28-d treatment with L-796568, a novel β3-adrenergic receptor agonist, on energy expenditure and body composition in obese men. American Journal of Clinical Nutrition 76, 780-8 (2002).

177. Bujalska IJ, Kumar S, Stewart PM. 1997 Does central obesity reflect “Cushing’s disease of the omentum?” Lancet. 349:1210–1213.

178. Walker, B.R., Connacher, A.A., Lindsay, R.M., Webb, D.J. & Edwards, C.R.W. Carbenoxolone increases hepatic insulin sensitivity in man: A novel role for 11-oxosteroid reductase in enhancing glucocorticoid receptor activation. Journal of Clinical Endocrinology and Metabolism 80, 3155-9 (1995).

179. Sandeep, T.C., Andrew, R., Homer, N.Z.M., Andrews, R.C., Smith, K. & Walker, B.R. Increased in vivo regeneration of cortisol in adipose tissue in human obesity and effects of the 11β-hydroxysteroid dehydrogenase type 1 Inhibitor carbenoxolone. Diabetes 54, 872-9 (2005).

180. Liu J , Wang L , Zhang A , Di W , Zhang X , Wu L , Yu J , Zha J , Lv S , Cheng P , Hu M , Li Y , Qi H , Ding G , Zhong Y . Adipose tissue-targeted 11β-hydroxysteroid dehydrogenase type 1 inhibitor protects against diet-induced obesity. Endocr J. 2011;58(3):199-209.

181. Rupnick MA , Panigrahy D , Zhang CY , Dallabrida SM , Lowell BB , Langer R , Folkman MJ . Adipose tissue mass can be regulated through the vasculature. Proc Natl Acad Sci U S A. 2002 Aug 6;99(16):10730-5.

182. Kim YM , An JJ , Jin YJ , Rhee Y , Cha BS , Lee HC , Lim SK . Assessment of the anti-obesity effects of the TNP-470 analog, CKD-732. J Mol Endocrinol. 2007 Apr;38(4):455-65.

183. AngioLab’s candidates. Available from: http://angiolab.co.kr/

184. Koubova J , Guarente L . How does calorie restriction work? Genes Dev. 2003 Feb 1;17(3):313-21.

185. Blüher M , Kahn BB , Kahn CR . Extended longevity in mice lacking the insulin receptor in adipose tissue. Science. 2003 Jan 24;299(5606):572-4.

186. Frédéric Picard, Martin Kurtev, Namjin Chung, Acharawan Topark-Ngarm, Thanaset Senawong, Rita Machado de Oliveira, Mark Leid, Michael W. McBurney, and Leonard Guarente. Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-γ. Nature. 2004 June 17; 429(6993): 771.

187. Lagouge, M. et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1[alpha]. Cell 127, 1109-22 (2006).

188. Feige, J.N. et al. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metabolism 8, 347-58 (2008).

189. Sirtris pipeline. Available from: http://www.sirtrispharma.com/pipeline.html

190. Cummings DE, Overduin J, Foster-Schubert KE. Gastric bypass for obesity: mechanisms

of weight loss and diabetes resolution. *J. Clin. Endocrinol. Metab.* 89(6), 2608–2615 (2004).

191. Cummings DE, Shannon MH. Roles for ghrelin in the regulation of appetite and body weight. *Arch. Surg.* 138(4), 389–396 (2003).

192. Vincent RP, Le Roux CW. The satiety hormone peptide YY as a regulator of appetite. *J. Clin. Pathol.* 61(5), 548–552 (2008).

193. Batterham RL, Cohen MA, Ellis SM *et al.* Inhibition of food intake in obese subjects by peptide YY3–36. N. Engl. J. Med. 349(10), 941–948 (2003).

194. Le Roux CW, Welbourn R, Werling M et al. Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. Ann. Surg. 246(5), 780–785 (2007).