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Anti-Obesity Therapy: from Rainbow Pills to Polyagonists

T. D. Müller, C. Clemmensen, B. Finan, R. D. DiMarchi, and M. H. Tschöp

Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany (T.D.M., C.C., M.H.T.); German Center for Diabetes Research, Neuherberg, Germany (T.D.M., C.C., M.H.T.); Department of Chemistry, Indiana University, Bloomington, Indiana (B.F., R.D.D.); and Division of Metabolic Diseases, Technische Universität München, Munich, Germany (M.H.T.)

Address correspondence to: T. D. Müller, Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, Parkring 13, 85748 Garching, Germany. E-mail: timo.mueller@helmholtz-muenchen.de; or M. H. Tschöp, Institute for Diabetes and Obesity, Helmholtz Diabetes Center, Helmholtz Zentrum München, Parkring 13, 85748 Garching, Germany. E-mail: matthias.tschoep@helmholtz-muenchen.de

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Abstract*——With their ever-growing prevalence, obesity and diabetes represent major health threats of our society. Based on estimations by the World Health Organization, approximately 300 million people will be obese in 2035. In 2015 alone there were more than 1.6 million fatalities attributable to hyperglycemia and diabetes. In addition, treatment of these diseases places an enormous burden on our health care system. As a result, the development of pharmacotherapies to tackle this life-threatening pandemic is of utmost importance. Since the beginning of the 19th century, a variety of drugs have been evaluated for their ability to decrease body weight and/or to improve deranged glycemic control. The list of evaluated drugs includes, among many others, sheep-derived thyroid extracts, mitochondrial uncouplers, amphetamines, serotonergics, lipase inhibitors, and a variety of hormones produced and secreted by the gastrointestinal tract or adipose tissue. Unfortunately, when used as a single hormone therapy, most of these drugs are underwhelming in their efficacy or safety, and placebo-subtracted weight loss attributed to such therapy is typically not more than 10%. In 2009, the generation of a single molecule with agonism at the receptors for glucagon and the glucagon-like peptide 1 broke new ground in obesity pharmacology. This molecule combined the beneficial anorectic and glycemic effects of glucagon-like peptide 1 with the thermogenic effect of glucagon into a single molecule with enhanced potency and sustained action. Several other unimolecular dual agonists have subsequently been developed, and, based on their preclinical success, these molecules illuminate the path to a new and more fruitful era in obesity pharmacology. In this review, we focus on the historical pharmacological approaches to treat obesity and glucose intolerance and describe how the knowledge obtained by these studies led to the discovery of unimolecular polypharmacology.

I. Introduction

The escalating prevalence of diabetes and obesity represents an incessant and escalating burden to modern societies. Obesity is characterized by an excess of body fat resulting from a chronic surplus in energy intake over energy expenditure. In the progression of obesity, the lipid deposition in adipose tissue can exceed the storage capacity of adipocytes, resulting in elevated circulating concentrations and inappropriate accumulation in multiple tissues, most notably liver and skeletal muscle. Fat deposits in such ectopic tissues are unhealthy and can initiate tissue inflammation, endoplasmic reticulum (ER) stress, and endothelial dysfunction, accelerating the development of obesity-associated pathologies, such as insulin resistance and type 2 diabetes (T2D) (Hotamisligil et al., 1993, 1996; Ozcan et al., 2004). In line with this proposed model of lipotoxicity, ectopic accumulation of reactive lipid species such as diacylglycerol, free fatty acids, free cholesterol, and ceramides have all been demonstrated to impair systems metabolism through local tissue inflammation and induction of ER stress (Unger, 2002; Virtue and Vidal-Puig, 2008; Symons and Abel, 2013; Contreras et al., 2014).

Of the numerous comorbidities linked to obesity, the most common are T2D; a variety of cardiovascular complications such as hypertension, coronary artery disease, and stroke; and certain types of cancer (Guo and Garvey, 2016). Notably, obesity and T2D represent top preventable causes of premature death and disability (Mathers and Loncar, 2006; Bauer et al., 2014), and the World Health Organization estimates that annually approximately 1.5 million deaths are directly attributable to diabetes (WHO, 2016). In the United States alone, about a quarter of a million adults prematurely die every year due to the consequences of excess body weight (Allison et al., 1999). The global burden that obesity and diabetes places upon our health care systems demands the development of effective, safe, and sustainable treatment options to combat this sizable and growing public dilemma.

ABBREVIATIONS: 5HT, serotonin; AgRP, agouti-related peptide; Aib, aminoisobuturic acid; CB1R, type I cannabinoid receptor; CEX, C-terminal extension; CNS, central nervous system; DIO, diet-induced obese; dn, dominant-negative; DNP, 2,4-dinitrophenol; DPP-IV, dipeptidylpeptidase IV; E2, estradiol; ER, endoplasmic reticulum; FDA, Food and Drug Administration; FGF21, fibroblast growth factor 21; GcgR, glucagon receptor; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; HFD, high-fat diet; LDL, low-density lipoprotein; NPY, neuropeptide Y; OXM, oxyntomodulin; PEG, polyethylene glycol; phen-fen, phentermine-fenfluramine; POMC, proopiomelanocortin; PYY, peptide YY; RYGB, Roux-en-Y gastric bypass; T2D, type 2 diabetes; T3, tri-iodothyronine; TR, thyroid receptor; TRb, β receptor; VSG, vertical sleeve gastrectomy.

In 1938, the United States passed the Food, Drug, and Cosmetic Act, upon which manufacturers were compelled to document drug safety to the Food and Drug Administration (FDA) to acquire approval for marketing and distribution. In 1962, through congressional approval of the Kefauver–Harris amendment, the FDA was authorized to review drug efficacy, and as such rendered the agency the final arbiter of the risks and benefits supporting drug approval and distribution in the United States (Colman, 2005). The drug approval process directed by the FDA or the European Medicines Agency has continually evolved and is currently separated into several distinct clinical phases. Phase I is typically performed in healthy volunteers with a specific focus on tolerability, pharmacokinetics, and acute measures of safety. Phase II progresses to assess drug efficacy and safety in the first cohorts of carefully selected and well-characterized patients. The phase III clinical studies aim to confirm sustained efficacy and longer-term safety, in large-scale multicenter patient trials. Once a drug is registered yet subsequent to commercialization, phase IV studies are often employed to further assess effects in even larger-scale, chronic studies. From start to finish, the governmental regulatory agencies assess and monitor the risk–benefit of drug candidates and registered medicines, with authority to restrict or remove them in clinical use.

Although regulatory oversight is well defined, the historical performance as it pertains to drugs controlling body weight has been populated with notable challenges (Colman, 2005). Many weight-lowering pharmacotherapies that were initially approved for treatment of obesity were subsequently withdrawn as safety concerns emerged to dominate the pharmacological benefits (Astrup, 2010; Adan, 2013). Whereas unfavorable balance in safety relative to efficacy determined the fate of several highly promising pharmacotherapies, weight loss induced by bariatric surgery has proven remarkably effective and sustainable. The improvement of metabolic control following a surgical intervention cannot be singularly justified by the physical limitation in gastrointestinal uptake of food, and notably glucose metabolism is typically improved much before a meaningful decrease in body weight is observed (Pories et al., 1995; Peterli et al., 2009; Bayham et al., 2012). Because the improvement in metabolism following surgical intervention relies on endocrine factors, it provides optimism for the discovery of medicinal options to counteract excess body weight in comparable magnitude to invasive surgery, but only time will tell whether this is a "mission impossible."

Although historical weight-loss drugs failed to meet expectations (Fig. 1), there has been important progress in recent years in the emergence of novel therapeutics. In particular, peptide-based agonism at the glucagon-like peptide 1 (GLP-1) receptor (GLP-1R) has demonstrated meaningful reduction in body weight and serves as a central ingredient to which additional pharmacology of other key metabolic hormones has been integrated to single molecular entities. Several purposefully designed, unimolecular multiagonists have recently been reported, with the first occurring in 2009 (Day et al., 2009). Nearly every pharmaceutical company active in cardiometabolic diseases is pursuing some aspect of this conceptual approach (Brandt et al., 2018). In this review, we present the achievements and the disappointments in modern weight-loss pharmacology and discuss the emergence of these unimolecular multiagonists as a promising path to a new era.

II. Bariatric Surgery: A Benchmark for Efficacy

As of today, bariatric surgery remains the most effective way to sizably lower body weight. Among the commonly used procedures are Roux-en-Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG), or adjustable gastric banding. The continued refinement through the last decade in surgical techniques and improvement in laparoscopic procedures has resulted in enhanced recovery, fewer adverse outcomes, and hospitalization routinely required for typically no more than 1 to 2 days (Robinson, 2009).

Bariatric surgery is rapidly gaining in popularity, and large-scale follow-up studies dependent on the surgical procedure, demonstrating sustained weight loss of 13%–27%, with follow-up for as much as 15 years (Sjöström et al., 2007). The body weight loss following RYGB is beneficially accompanied by a decrease in subsequent mortality (Sjöström et al., 2007) and often the complete remission of insulin resistance and T2D (Pories et al., 1995; Carlsson et al., 2012). This last observation has led the American Diabetes Association to recommend bariatric surgery in certain circumstances also for the treatment of T2D (Chakradhar, 2016; Rubino et al., 2016).

Rapid improvement in glycemic control after RYGB or VSG is observed within a few days following surgery, and notably prior to clinically relevant weight loss (Pories et al., 1995; Peterli et al., 2009; Bayham et al., 2012). About 8 weeks after the surgery, approximately 80% of patients are able to discontinue diabetes medication (Bayham et al., 2012), with improved insulin sensitivity demonstrated within the first postoperative week (Isbell et al., 2010; Umeda et al., 2011; Jørgensen et al., 2012; Faria et al., 2013). Some case reports show a decrease in fasting blood glucose of more than 40% by the end of the first postoperative day, with resolution of T2D and discontinuation of exogenous insulin treatment after just 6 days (Pories et al., 1995).

The molecular mechanisms underlying the sustained improvement in body weight and glucose metabolism following bariatric surgical interventions are not completely understood and are subject of intense scientific investigation. Despite some contention

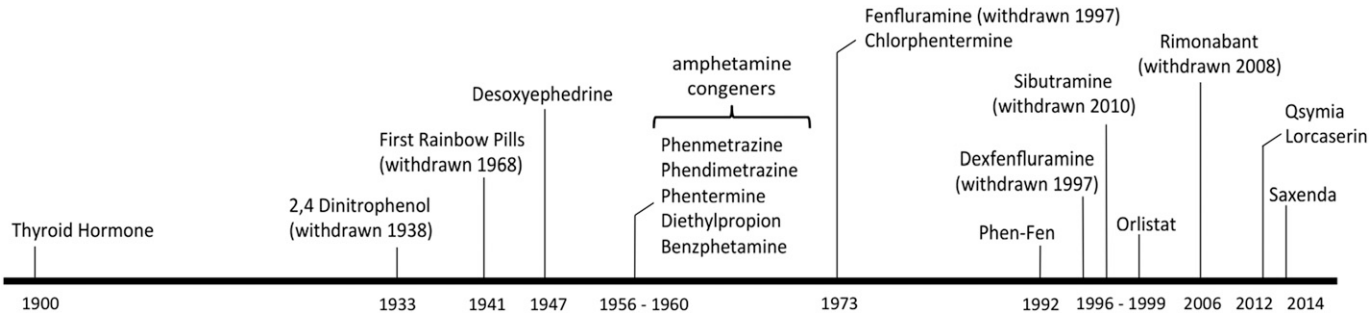

Fig. 1. Time line of pharmacotherapies used to treat obesity from 1900 until today.

(Werling et al., 2013, 2015), changes in energy expenditure or the rate by which nutrients are taken up from the intestine do not explain the initial efficacy or the sustained weight reduction (Olbers et al., 2006; Odstrcil et al., 2010; Carswell et al., 2014; Münzberg et al., 2015; Schmidt et al., 2016). Chronic changes in food consumption are typically reported after bariatric surgery and are considered a causal factor in sustained weight loss (Brolin et al., 1994; Sjöström et al., 2004; Laurenius et al., 2012; Münzberg et al., 2015). Molecular mechanisms that support reduction in food intake seem, however, not to rely on physical limitations of the gastrointestinal tract (Ryan et al., 2014), but instead pertain to changes in food preference, taste perception, and alterations in the food reward system (Scruggs et al., 1994; Burge et al., 1995; Miras and le Roux, 2010; Shin and Berthoud, 2011; Mathes and Spector, 2012; Laurenius et al., 2013).

Endocrine changes in gastrointestinal hormone secretion following RYGB or VSG include enhanced postprandial secretion of GLP-1 (le Roux et al., 2006; Korner et al., 2007; Isbell et al., 2010; Jacobsen et al., 2012; Bojsen-Moller et al., 2014; Svane et al., 2016) and peptide YY (PYY) (le Roux et al., 2006; Svane et al., 2016), whereas circulating levels of glucose-dependent insulinotropic polypeptide (GIP) and ghrelin are typically diminished (Cummings et al., 2002; Korner et al., 2007). The observation that RYGB and VSG lead increased postprandial GLP-1 secretion has widely resulted in the hypothesis that enhanced GLP-1 action contributes to reduced food intake, weight loss, and improved glucose metabolism typically observed after these surgical procedures. However, VSG also improves body weight and glycemic control in mice deficient for the GLP-1 receptor, and surprisingly with equal efficiency when compared with wild-type controls (Wilson-Perez et al., 2013). These data suggest that enhanced GLP-1 action alone cannot explain the metabolic benefits of this surgery. Consistent with this notion, singular inhibition of either GLP-1 or PYY alone does not affect food intake in humans following RYGB (Svane et al., 2016). However, as shown in the same study, when GLP-1 and PYY signaling are both collectively blocked, food consumption is increased by ~20%. Consequently, it has been proposed repeatedly that GLP-1, when acting in concert with other gastrointestinal peptides, might play a role in the sustained weight loss associated with bariatric surgery (Svane et al., 2016). Notably, also signaling via the farnesoid-X-activated receptor seems to play an important role in the metabolic benefits achieved by bariatric surgery because the efficacy of VSG to decrease body weight is strikingly reduced in mice lacking farnesoid-X-activated receptor (Ryan et al., 2014).

In summary, whereas the molecular mechanisms underlying the improvement in energy and glucose metabolism by bariatric surgery are not fully understood, surgery is the only currently available intervention that achieves sustained weight loss and correction of T2D. However, with the appreciable sustained efficacy, it should be noted that a surgical approach to disease management is highly invasive, irreversible in most instances, and not without risk (Marcotte and Chand, 2016). In most countries, bariatric surgery is accessible to only a small fraction of patients who are extremely obese. In this regard, the National Institutes of Health recommends bariatric surgery for obesity at a body mass index >40 kg/m^2 or at a body mass index >35 kg/m^2 in association with other significant comorbidities requiring an immediate weight reduction (Robinson, 2009). Consequently, it remains an important option for extreme forms of obesity, but surgery seems impractical as a general strategy to tackle the global "diabesity" pandemic. Accordingly, pharmacological options to similarly address excess body weight and improve glycemic control are urgently required.

III. The Chronology of Modern Weight-Loss Pharmacology

Modern weight-loss pharmacology begins near the end of the 19th century, where growing industrialization and change in lifestyle propelled a persistently increasing demand for drugs to control body weight (Fig. 1). Eager to satisfy a generation that was yearning for a pill to melt away fat mass within a (few) blink(s) of an eye, a variety of weight-lowering drugs were evaluated during the first half of the 20th century (Rodgers et al., 2012; Adan, 2013). Reflective of the complex

nature of systemic energy regulation, the mode of action underlying these drugs ranged from central modulation of hunger and satiety, to limiting intestinal nutrient transport, and to direct manipulation of energy expenditure. Viewed retrospectively, certain of these pharmacological approaches could easily be characterized as careless and unmindful. Additionally, some of the people using them were equally so in their quest for supplemental beauty.

A. Thyroid Hormones

The thyroid gland has been known for centuries for its ability to increase metabolic rate. There are clinical reports that date from the 1890s on the weight-lowering effect of sheep-derived thyroid extracts (McCone, 1897). Apart from decreasing body weight and body fat mass via stimulation of energy expenditure, thyroid hormone also improves hepatic lipid metabolism. It can decrease low-density lipoprotein (LDL) cholesterol via enhanced reverse cholesterol transport and clearance of LDL via the liver (Baxter and Webb, 2009). Unfortunately, excess thyroid hormone action also leads to muscle and bone catabolism, as well as to cardiac arrhythmia, tachycardia, and heart failure, which severely limits its use to control body weight (von Olshausen et al., 1989; Woeber, 1992; Galloe et al., 1993). Nevertheless, at the end of the 19th century, physicians already experimented with thyroid extracts to lower body weight and occasionally prescribed strychnine or digitalis leaf to attenuate thyroid hormone's adverse cardiovascular effects (Cohen et al., 2012). Regrettably, in some cases, excessive thyroid hormone supplementation proved a terminal treatment and served to accentuate that "all that glitters, is not gold" (Bhasin et al., 1981).

The biologically active form of thyroid hormone is tri-iodothyronine (T3), which promotes its pharmacology through two specific nuclear thyroid receptor (TR) isofroms, TRα and TRβ. Notably, T3 stimulation of heart rate is substantially impaired in mice lacking TRα, whereas the ability of T3 to lower cholesterol is fully preserved in these mice (Grover et al., 2003). Relative to wild-type controls, T3 stimulation of metabolic rate is only blunted by about 50% in mice lacking TRα. Treatment of rats and nonhuman primates with the TRβ-selective agonist KB-141 further increased metabolic rate and decreased cholesterol and body weight, without affecting heart rate (Grover et al., 2003). Collectively, these data suggest that T3 regulates cholesterol metabolism via signaling through TRβ, whereas the cardiovascular effects of T3 are mediated via TRα, and T3 regulation of metabolic rate is mediated via both TRα and TRβ (Grover et al., 2003).

Administration of T3 increases metabolic rate in a variety of species, including mice (Oh and Kaplan, 1994; Jekabsons et al., 1999; Grover et al., 2003, 2005), rats (Whaley et al., 1959), and humans (Barbe et al., 2001; Johannsen et al., 2012). Notably, T3 also enhances oxygen consumption in excised rat tissues such as the liver, kidney, heart, and skeletal muscle (Whaley et al., 1959), indicating that T3 stimulation of metabolic rate is mediated through direct tissue action. The molecular mechanism underlying T3 modulation of metabolic rate includes uncoupling of oxidative phosphorylation from mitochondrial ATP synthesis in skeletal muscle and other peripheral tissues (Hess and Martius, 1951; Lardy and Feldott, 1951; Lebon et al., 2001), regulation of lipogenesis (Freake et al., 1989), activation of Na^+/K^+ ATPase (Izmail-Beigi and Edelman, 1970), enhanced mitochondrial biogenesis (Gustafsson et al., 1965), and stimulation of futile cycling (Newsholme and Crabtree, 1976; Shulman et al., 1985). In line with the proposed role of T3 to regulate energy metabolism via mitochondrial uncoupling in skeletal muscle, mRNA levels of uncoupling protein 3 are decreased threefold in skeletal muscle of hypothyroid rats and are increased sixfold in hyperthyroid rats relative to euthyroidic controls (Gong et al., 1997). Administration of T3 further increases expression of uncoupling protein 3 in skeletal muscle of various species, including mice (Jekabsons et al., 1999; Jucker et al., 2000), rats (Larkin et al., 1997; Masaki et al., 2000), and humans (Barbe et al., 2001). Although these and numerous other studies convincingly show that T3 affects metabolic rate via action in peripheral tissues, more recent studies suggest that T3 also affects energy metabolism via central nervous system (CNS)–dependent mechanisms that include hypothalamic regulation of 5′ adenosine monophosphate-activated protein kinase (Lopez et al., 2010; Martinez-Sanchez et al., 2017) and sympathetic nervous system–mediated regulation of brown adipose tissue (Alvarez-Crespo et al., 2016). However, no effect on energy expenditure or body weight was reported in another study in which T3 was chronically administered into either the paraventricular hypothalamus or the ventromedial hypothalamus of rats (Zhang et al., 2016).

In summary, case reports on the use of thyroid hormones to control excess body weight date back to the end of the 19th century (McCone, 1897). Due to its ability to lower body weight and to improve lipid metabolism, thyroid hormone also became one of the major active constituents of the famous rainbow pills, which are discussed later in this review (see *The Rainbow Pills*). However, the pharmacological potential of T3 is restricted by adverse effects, predominantly cardiovascular in nature. Nevertheless, the hepatic action of T3 to improve lipid metabolism renders T3 a validated candidate for more advanced pharmacological approaches. Consistent with this perspective, and as discussed later in this review, a novel strategy entails the recruitment of a peptide hormone to preferentially deliver T3 to the liver (Finan et al., 2016a).

B. 2,4-Dinitrophenol

During the First World War, a number of French munitions workers died after being accidentally exposed

to large amounts of 2,4-dinitrophenol (DNP), a substance that was used in the manufacture of explosives (Perkins, 1919). DNP was also commonly used as a dye, for wood preservation, photographic development, and as an herbicide (Harper et al., 2001; Grundlingh et al., 2011). An overdose of DNP results in overheating, fever, and eventually death (Perkins, 1919; Tainter et al., 1934). In the decade following 1910, more than 35 cases of fatal DNP intoxication were recorded (Horner, 1941; Grundlingh et al., 2011).

In 1933, Maurice Tainter from Stanford University published the first clinical report on the weight-lowering properties of DNP (Tainter et al., 1933). DNP was administered in a daily dose between 3 and 5 mg/kg, and the reported weight loss was in the range of 1.5 kg per week. The pharmacological effect resided in the ability of DNP to increase metabolic rate via enhanced mitochondrial uncoupling, thus favoring heat production over ATP synthesis (Cutting et al., 1933; Tainter et al., 1933, 1935). When used in a dose of 300 mg/d, weight loss induced by DNP seemed to be well tolerated and associated with an increase in metabolic rate of ~50% (Tainter et al., 1933, 1934; Dunlop, 1934). In subsequent clinical studies, metabolic rate was shown to increase in average by 11% per 100 mg DNP (Dunlop, 1934; Tainter, 1935; Harper et al., 2001). In anesthetized dogs, DNP dose-dependently increased oxygen consumption by as much as 12-fold above baseline (Hall et al., 1933). Although the weight-lowering effect of DNP is impressive, the margin between ED_{50} and LD_{50} is razor-thin. In line with this notion, acute administration of 20–50 mg/kg DNP can be lethal in humans (Macnab and Fielden, 1998; Hsiao et al., 2005), and its extended half-life that ranges from 46 hours in mice (Robert and Hagardorn, 1985) to 54–88 hours in humans (Zhao et al., 2015) further complicates its use. Repeated daily administration of DNP leads to accumulation, and with it the risk for fatal intoxication is increased. In line with this notion, early clinical studies showed that metabolic rate gradually increases with daily administration of 3–5 mg/kg DNP, and plateaued at ~40% above baseline after a few weeks of treatment (Cutting et al., 1933; Harper et al., 2001). Following its introduction as a weight loss pharmacotherapy in 1933, the interest in DNP was tremendous. In 1934 alone, Stanford Clinics supplied over 1,200,000 DNP capsules to physicians, or directly to patients with physician prescriptions (Tainter et al., 1934). Stanford scientists estimated that within this 1 year, more than 100,000 people in the United States were treated with DNP.

The classic adverse effects of DNP include hyperthermia, tachycardia, diaphoresis, tachypnea, nausea, and vomiting (Grundlingh et al., 2011). Nevertheless, commercialization of DNP boomed until reports of adverse liver, heart, and muscle effects (MacBryde and Taussig, 1935) prompted the FDA in 1935 to state that "treatment of a mild chronic condition such as obesity with a toxic agent capable of inducing serious injury and death appears unjustified" (Colman, 2007). The FDA, however, did not suspend DNP until 1938, when reports accumulated that linked the administration of DNP, even when administered in physiologic doses and under physician supervision, to the development of cataracts (Horner et al., 1935; Horner, 1936; Colman, 2007). Until the removal from commercial distribution in 1938, it is estimated that more than 2500 Americans were blinded as a result of DNP-induced cataracts (Horner, 1936; Colman, 2007).

The use of DNP did not completely disappear despite termination in 1938 (Fig. 1). In 1981, a United States physician restarted commercializing DNP in self-made diet pills (Mitcal), which he distributed as intracellular hyperthermia therapy in his private weight-loss clinic (Kurt et al., 1986; Grundlingh et al., 2011). In 1982, the FDA received notice from patients using Mitcal, and in a subsequent lawsuit it was estimated that approximately 14,000 people had been treated, with one reported fatality. After being fined in 1986 for drug law violations, this physician continued prescribing DNP for various clinical applications until 2008 when he was sentenced for trading DNP as an intracellular hyperthermia cancer therapy (Grundlingh et al., 2011). Despite this dubious history, DNP remains a common illegal drug substance employed by bodybuilders and others who are either careless or misguided in risking their health to melt away fat.

C. Amphetamines

1. Methamphetamine. In the 1940s, amphetamines gained rapid popularity given their appetite-suppressing effect, and the FDA approved the use of methamphetamine desoxyephedrine (tradenames Hydrin and Desoxyn) for the treatment of obesity in 1947 (Colman, 2005) (Fig. 1). The appetite suppression of methamphetamine (well recognized by its street name crystal meth) resides in its ability to stimulate the synthesis and release of catecholamines, especially dopamine, from CNS nerve terminals. This leads to an increase in metabolic rate and stimulation of anorectic hypothalamic neurocircuits and other brain areas. In the hypothalamus, monoaminergic neurons project from the arcuate nucleus to the median eminence, and amphetamine stimulation of monoamine release inhibits food intake via stimulation of proopiomelanocortin (POMC) neuronal activity, whereas neurons expressing neuropeptide Y (NPY)/agouti-related peptide (AgRP) are inhibited (Heisler et al., 2002, 2006; Kuo, 2005, 2006; Garfield and Heisler, 2009; Kuo et al., 2009, 2012; Roepke et al., 2012; Chu et al., 2014; Jones and Bloom, 2015).

The interest in using amphetamines for the purpose of controlling excess body weight was propelled by case reports showing that thrice daily administration of 2 mg desoxyephedrine could decrease body weight by as

much as 24.5 kg, and seemingly without major adverse effects (Ray, 1947). Nonetheless given their action on the central reward system, amphetamines possess a certain risk for addiction (Schuster and Thompson, 1969; Balster and Schuster, 1973; Yokel and Pickens, 1973; Gotestam and Andersson, 1975). The use of desoxyephedrine for body weight lowering was not the intended initial purpose, which was treatment of narcolepsy, depression, postencephalitic Parkinson syndrome, alcoholism, cerebral arteriosclerosis, and hay fever (Colman, 2005). The addictive and abuse potential of desoxyephedrine hampered its pharmacological use to treat obesity and inspired pharmaceutical companies to develop chemically-related analogs that were intended to retain the anorectic effect, but with far less safety concerns. Up until 1960, several amphetamine congeners, such as phenmetrazine, phendimetrazine, diethylpropion, phentermine, and benzphetamine, were registered by the FDA for use as an adjunct to lifestyle change in the treatment of obesity (Colman, 2005) (Fig. 1).

2. Amphetamine Congeners. Like other amphetamines, the congeners act as sympathomimetics to stimulate the release of monoamines, especially dopamine and/or norepinephrine from CNS nerve terminals. The purportedly reduced addictive potential of the congeners remained in question (Wilson et al., 1971; Gotestam and Andersson, 1975), and the FDA, after the Kefauver–Harris amendments became effective in 1962, more closely scrutinized the risk and benefits. A large meta-analysis comprising more than 200 clinical studies and more than 10,000 individuals concluded that the congeners, although statistically significant in lowering body weight, are only marginally more effective relative to treatment with placebo (Colman, 2005). Of note, the FDA at that point in history defined the efficacy of a drug as "statistically different (superior) to placebo," unfortunately neglecting whether the observed difference was of clinical relevance. Consequently, despite showing only limited efficacy, the congeners remained approved drugs for the treatment of obesity. Because a certain addictive potential could not be ruled out (Gotestam and Andersson, 1975), the FDA henceforth restricted their use to only short-term (a few weeks) treatment (Colman, 2005). The limited efficacy paired with restrictions for use largely dampened the pharmacological interest in amphetamine congeners as monotherapy for body weight management. However, some congeners still remain approved in combination to lifestyle modifications for the short-term treatment of obesity and are, as later reviewed, used in adjunct to other weight loss drugs.

a. Phenmetrazine. Phenmetrazine was approved by the FDA for the treatment of obesity in 1956 and was commercialized under the trade name Preludin (Boehringer-Ingelheim, Ingelheim, Germany) (Colman, 2005). Preludin has sympathomimetic properties similar to that of ephedrine and amphetamine and thus inhibits food intake by stimulating the release of norepinephrine and dopamine from CNS nerve terminals (Ressler, 1957; Szenas and Pattee, 1957; Leith and Beck, 1958; Rothman and Baumann, 2006). Preludin was commonly administered thrice daily in an amount of 25 mg, and, when given in adjunct to a calorie-restricted diet, placebo-subtracted weight loss induced by preludin is typically about ~0.5 kg/wk with significant improvement of blood glucose after 4–6 weeks of treatment (Robillard, 1957; Leith and Beck, 1958; Briggs et al., 1960; Baggio et al., 2004). Adverse effects associated with phenmetrazine include tachycardia, heart arrhythmias, hypertension, convulsions, restlessness, agitation, vomiting, and diarrhea (Clarke, 2007). Due to its action on the brain reward system, phenmetrazine still carries an abusive potential (Bethell, 1957) and when chronically used can lead to delusions and paranoia (Clarke, 2007). There is at least one reported case of fatal phenmetrazine poisoning (Norheim, 1973). Due to its euphoric effect, phenmetrazine enjoyed great popularity in the mid 1950s and was misused for recreational purposes before its commercialization was discontinued.

b. Phendimetrazine. Phendimetrazine is a prodrug of phenmetrazine and was approved by the FDA for the short-term treatment of obesity in 1959 (Fig. 1). The prodrug character of phendimetrazine is based on the addition of a methyl group onto an amphetamine backbone. This chemical modification renders the drug inactive unless the methyl group is cleaved in the circulation and phendimetrazine is converted to phenmetrazine. Because only a portion of the drug is active after being administered into the circulation, phendimetrazine has a lower abusive potential relative to phenmetrazine. Phendimetrazine is usually given orally and has a half-life of 2–4 hours in humans. When given in a daily dose of 210 mg (6×35 mg), weight loss induced by phendimetrazine is about 7% relative to the baseline body weight after up to 32 weeks of treatment (Le Riche and Van Belle, 1962). Unfortunately, no placebo group was included in this study (Le Riche and Van Belle, 1962). A dose of 210 mg/d is reported to be generally well tolerated, with insomnia, dry mouth, and constipation being the most frequently reported side effects. Less frequently reported acute side effects include hyperpyrexia, mydriasis, chest pain, arrhythmias, delirium, and rhabdomyolysis (Kwiker et al., 2006), whereas more chronic adverse effects include the development of cardiomyopathies (Rostagno et al., 1996; Landau et al., 2008). As of today, phenmetrazine is approved by the FDA in adjunct to lifestyle changes for the short-term treatment of obesity and is commercialized under the trade name Adipost, Anorex-SR, Appecon, Bontril PDM, Melfiat, Obezine, Phendiet, Plegine, Prelu-2, or Statobex.

c. Diethylpropion. Diethylpropion (a.k.a. amphepramone) was approved by the FDA in 1959 as adjunct

to diet and exercise for the short-term (<12 weeks) treatment of obesity (Fig. 1). Diethylpropion is structurally related to bupropion and is commercialized under the trade name Tenuate. Diethylpropion is usually given either thrice daily in an amount of 25 mg, or once daily as an extended release formulation. As demonstrated in a recent meta-analysis comprising 25 clinical studies, placebo-subtracted weight loss attributed to treatment with diethylpropion is on average 1.28 kg when given for <180 days and 6.5 kg when given >180 days (Lucchetta et al., 2017). In a more historic meta-analysis comprising 13 clinical studies published between 1965 and 1983, treatment with diethylpropion for 6–52 weeks in a dose of 75 mg/d resulted in a placebo-subtracted average weight loss of 3.0 kg with borderline significance to placebo controls (Li et al., 2005). Notably, although several reports testify that diethylpropion has an anorexigenic nature with virtual absence of adverse effects (Wilson and Long, 1960; Hadden and Lucey, 1961; Nash, 1961; Seaton et al., 1961), there is at least one report of diethylpropion addiction (Clein and Benady, 1962).

d. Phentermine. Phentermine was approved by the agency for the treatment of obesity in 1959. The duration of treatment is restricted to 12 weeks and is indicated to be used in adjunct to lifestyle modifications. Phentermine is a sympathomimetic with agonism at the trace amine-associated receptor 1. The drug stimulates primarily the release of norepinephrine, but to lower extent also dopamine and serotonine. Depending on the dose (which is typically between 30 and 37.5 mg/d) the placebo-subtracted weight loss attributed to treatment with phentermine (30 mg/d) is in the range of 4–8 kg after 12 weeks of treatment (Kim et al., 2006; Kang et al., 2010; Moldovan et al., 2016). Phentermine is generally well tolerated with little to no abusive potential, even when used chronically for up to 21 years (Hendricks et al., 2009). In a large-scale survey, United States physicians stated that 98% use pharmacological options to treat obesity, and, from those physicians using pharmacotherapies, 97% state to prescribe phentermine, 64% diethylpropion, 60% phendimetrazine, 50% topiramate, 49% sibutramine, and 43% orlistat (Hendricks et al., 2009).

D. The Rainbow Pills

Undiscouraged by the failure of amphetamines and thyroid hormone as stand-alone treatment of obesity, the pharmaceutical industry has long been in search for a proverbial silver bullet to fight obesity. In 1941, Clark & Clark (Camden, NY) combined the anorectic effect of amphetamines with the thermogenic effect of thyroid hormone to form Clarkotabs, possibly the first commercially distributed polypharmacological diet pills (Fig. 1). Because Clarkotabs came in all sorts of seemingly harmless colors, they became popularized as the rainbow pills. The different colors were commonly misused to pretend their utility as personalized medicine, with the different colors reflecting individual patient need to optimally lose body weight. The first preparations included, in addition to thyroid hormones and amphetamine sulfate, aloin and atropine sulfate to counteract adverse cardiovascular effects (Cohen et al., 2012). The ingredients as well as the primary commercial manufacturer of the rainbow pills changed with time, and the different formulations often constituted a physical cocktail of weight-reducing substances, including d-amphetamine or related analogs (like diethylpropion, fenfluramine, sibutramin, or fenproporex), thyroid hormones, diuretics, laxatives, chlorthalidon, ephedrine, and/or phenolphthalein. Substances such as digitalis, belladonna, benzodiazepines, barbiturates, corticosteroids, cardiac glycosides, beta-blocker, and potassium were common additives used to counteract or mask adverse cardiovascular effects of the drug cocktail (Cohen et al., 2012).

In the decades between the 1940s and 1960s, the rainbow pills enjoyed great popularity. In contrast to good medical practice, the pills were often sent directly from the manufacturer to physicians, who then sold the pills to patients. Reflecting the lucrative financial potential in such therapy, at least 2000 United States clinical practices in 1967 focused exclusively on weight reduction (Cohen et al., 2012). According to a congressional investigation, it was estimated that these weight loss clinics earned annually ~$250 million in patient fees, and another $120 million was annually spent by patients to acquire the rainbow pills (Cohen et al., 2012). In 1968, a journalist for *Life* magazine reported on her experience in 10 weight loss clinics where she was prescribed more than 1500 pills, with only superficial counseling and without clear medical necessity to lose body weight (McBee, 1968). The FDA received notice of several fatalities linked to the consumption of rainbow pills and studied the process by which rainbow pills were commercialized (Henry, 1967). A subsequent large-scale investigation by the U.S. Senate ascribed more than 60 deaths to the consumption of rainbow pills, which prompted the FDA in 1968 to seize large quantities of rainbow pills from manufacturers and to prohibit further distribution in the United States (Cohen et al., 2012).

E. Serotonergics

1. Fenfluramine. Although federal restrictions largely dampened the clinical interest in the use of amphetamines to treat obesity during the decade of the 1960s, the interest in amphetamines was suddenly revived in 1973 when the first serotonergic, fenfluramine, received approval for the treatment of obesity (Fig. 1).

Fenfluramine promotes its anorectic action via stimulating the release of serotonin (5HT) from CNS neurons, while at the same time inhibiting its axonal reuptake

(Costa et al., 1971; Garattini, 1981; Garattini et al., 1986). Identified in 1948 for its vasoconstrictive action (Rapport et al., 1948a,b), 5HT is a key central neurotransmitter, and as such is implicated in a myriad of metabolic functions that include the regulation of mood, behavior, and food intake, among many others (Bello and Liang, 2011). In line with a role of 5HT in regulating energy intake, stimulation of postsynaptic 5HT receptors decreases food intake (Samanin et al., 1980), whereas in rats central 5HT depletion via intracerebroventricular administration of p-chlorophenylalanine results in overeating and obesity (Breisch et al., 1976). The anorectic action of 5HT is mediated via at least two central 5HT receptors, 5HT2c and 5HT1b, which are located on neurons expressing either POMC/cocaine- and amphetamine-regulated transcript (CART) or AgRP/NPY (Garfield and Heisler, 2009). The current consensus of 5HT-induced inhibition of food consumption includes binding of 5HT to the 5HT2c receptor on POMC/CART neurons with the result being activation (depolorization) of these neurons (Heisler et al., 2002), whereas at the same time neurons expressing AgRP/NPY get inactivated (hyperpolarized) via binding of 5HT to the 5HT1b receptor (Heisler et al., 2006; Garfield and Heisler, 2009).

Fenfluramine inhibits food intake in a variety of species, including rodents, guinea pigs, dogs, and humans (Alphin and Ward, 1969; Pinder et al., 1975). In line with the melanocortinergic system playing a key role in orchestrating this effect, mice lacking either the 5HT1b receptor (Lucas et al., 1998) or the melanocortin 4 receptor are unresponsive to the anorectic effect of fenfluramine (Heisler et al., 2006). Of appreciable note, unlike other amphetamines, fenfluramine, when given at anorectic doses, does not affect locomotor activity (Ledouarec and Schmitt, 1964; Garattini, 1981; Lucas et al., 1998) and is largely devoid of abusive potential (Gotestam and Andersson, 1975).

2. Phentermine-Fenfluramine. In 1992, the interest in using fenfluramine to lower body weight was promoted by a clinical report showing it potently lowers body weight when given as an adjunct to phentermine (Weintraub et al., 1992a). In this study, 121 obese individuals were treated for 34 weeks with a combination of 15 mg phentermine and 60 mg fenfluramine. The patients treated with this phentermine-fenfluramine (phen-fen) combination lost an average 14.2% body weight relative to 4.6% in placebo-treated controls (Weintraub et al., 1992a). Of appreciable note, dry mouth was the most common reported side effect, with all adverse features vanishing after 4 weeks of treatment (Weintraub et al., 1992a). Continuation of the study (but with varying study designs) for up to 210 weeks corroborated the overall metabolic benefits of the phen-fen combination and suggested that this pharmacotherapy, when given as an adjunct to lifestyle modifications, is of appreciable value for the treatment of obesity (Weintraub et al., 1992a,b,c,d). Of note, not all studies were able to show a superior effect of the phen-fen combination on body weight relative to treatment with phentermine or fenfluramine alone (Weintraub et al., 1984; Li et al., 2003). However, there are some indications that the phen-fen combination has a lower abuse potential relative to treatment with phentermine alone (Brauer et al., 1996). In 1996, it is estimated that phen-fen was prescribed to more than 18 million people in the United States (Connolly et al., 1997), and it was in the same year when the FDA approved the use of dexfenfluramine, the d-isomer of fenfluramine, as a chronic treatment of obesity (Colman, 2005) (Fig. 1).

3. Dexfenfluramine. When dexfenfluramine is given in a dose of 15 mg twice daily, placebo-subtracted weight loss attributed to dexfenfluramine is typically 3–6 kg, depending on the duration of treatment (Finer et al., 1988; Andersen et al., 1992; Geyer et al., 1995; Holdaway et al., 1995). The weight-lowering effect of dexfenfluramine has been confirmed in many, but not all clinical studies (Mathus-Vliegen, 1993; Recasens et al., 1995; Galletly et al., 1996). Beyond its ability to lower food intake via its action on the serotonergic/melanocortinergic system (Heisler et al., 2002), dexfenfluramine also lowers body weight independent of food intake, as animals treated with dexfenfluramine lose more body weight relative to mice that are pair-fed to receive the same amount of food as the dexfenfluramine-treated mice (Blundell et al., 1980). Furthermore, despite not being confirmed in every study (Pfohl et al., 1994), weight loss induced by dexfenfluramine is accompanied by a decrease in blood pressure, lower levels of cholesterol, and improvement of insulin resistance (Holdaway et al., 1995). The improvement in glycemic control following treatment with dexfenfluramine seems to be independent of weight loss because short-term administration of dexfenfluramine to obese patients improves glycemic control without affecting body weight (Andersen et al., 1993). However, shortly after dexfenfluramine was approved in 1996 by the FDA for the treatment of obesity, clinical reports emerged linking fenfluramine and dexfenfluramine to the development of pulmonary hypertension and valvular heart disease (Cannistra et al., 1997; Centers for Disease Control and Prevention (CDC), 1997; Connolly et al., 1997; Kurz and Van Ermen, 1997; Rasmussen et al., 1997). The increased prevalence of valvular regurgitation associated with the use of dexfenfluramine vanished 3–5 months after discontinuation of treatment (Weissman et al., 1999), but nevertheless prompted the manufacturer to voluntarily discontinue the commercialization of fenfluramine and dexfenfluramine in 1997 (Weissman et al., 1998).

4. Sibutramine. In 1997, the same year in which fenfluramine and dexfenfluramine were removed from the market, the FDA approved sibutramine, a serotonin and norepinephrine reuptake inhibitor, for the

treatment of obesity. In contrast to other monoamine reuptake inhibitors, sibutramine has only little clinical relevance as an antidepressant (Bello and Liang, 2011), but lowers body weight via inhibition of food intake and stimulation of energy expenditure (Heal et al., 1998; McNeely and Goa, 1998; Nelson and Gehlert, 2006; Astrup, 2010). Placebo-subtracted weight loss attributed to treatment with sibutramine is dependent on dose and treatment duration and typically resides in the range of 1.7–4.8 kg (1%–5%), but with significantly more patients achieving the 5% and 10% weight loss threshold relative to placebo-treated controls (Fujioka et al., 2000; McMahon et al., 2000; Smith et al., 2001; Wooltorton, 2002; Yanovski and Yanovski, 2002; Hauner et al., 2004; Rucker et al., 2007). Weight loss induced by sibutramine is accompanied by improvements in fasting insulin, triglycerides, and high-density lipoprotein cholesterol, but notably with an increase in blood pressure (Fujioka et al., 2000; McMahon et al., 2000; Wooltorton, 2002; Yanovski and Yanovski, 2002) and, even more strikingly, an increased risk for a cardiovascular event such as a cardiac arrhythmia (Wooltorton, 2002; James et al., 2010). The relatively mild weight loss achieved by sibutramine together with an increased risk of cardiovascular adverse effects led the FDA in 2010 to withdraw sibutramine from distribution.

F. Phentermine and Topiramate (Qsymia)

Although commercialization of fenfluramine was discontinued in 1997 due to cardiovascular adverse effects, phentermine, when used together with other weight-lowering agents, remains approved for the treatment of obesity. It was in 2012, when the FDA approved Qsymia, the combination of phentermine and topiramate as adjunct to lifestyle modification for the treatment of obesity (Fig. 1). Topiramate is a sulphamate-substituted monosaccharide derived from D-fructose (Privitera, 1997; Shank and Maryanoff, 2008; Edvinsson and Linde, 2010) and is commonly used to treat epilepsy (Langtry et al., 1997; Privitera, 1997) and migraine (Storey et al., 2001; Adelman et al., 2008; Edvinsson and Linde, 2010; Linde et al., 2013). The use of topiramate for these clinical applications seems to reside in its ability to selectively decrease CNS neuronal activity via inhibition of certain neuronal Ca^{2+} channels (Martella et al., 2008) as well as to modulate central glutamate and GABA signaling (White et al., 2000; Edvinsson and Linde, 2010). When used as monotherapy, placebo-subtracted weight loss induced by topiramate typically ranges between 3.8% and 6.5% depending on the dose (Aronne et al., 2013). The exact mechanism(s) of how topiramate improves systemic metabolism remains a subject of ongoing investigations. In any case, when given as an adjunct to phentermine, weight loss of this combination is greater as treatment with phentermine or topiramate alone (Aronne et al., 2013). In line with this notion, placebo-subtracted weight loss induced by the phentermine–topiramate combination is typically in the range of 5.9%–9.6% (Garvey et al., 2012, 2014a,b; Winslow et al., 2012; Aronne et al., 2013), and is associated with an improvement in glucose management (Garvey et al., 2012, 2014a) and the cardiovascular risk profile (Garvey et al., 2012, 2014b).

G. Orlistat

Orlistat is a lipase inhibitor that limits the availability of fatty acids for absorption by inhibiting gastrointestinal lipase activity (Drent et al., 1995). The resulting fat malabsorption facilitates a negative energy state leading to a placebo-subtracted weight loss in the range of 2.6% (Davidson et al., 1999; Khera et al., 2016). Orlistat has additional beneficial effects on glycemic control and nonalcoholic fatty liver disease (Hollander et al., 1998; Zelber-Sagi et al., 2006). The positive effects of orlistat on nonalcoholic fatty liver disease exceed what can be explained by changes in body weight alone (Zelber-Sagi et al., 2006). The glycemic benefits of orlistat can be potentiated by metformin coadministration (Miles et al., 2002). Most common adverse events are of gastrointestinal or digestive nature. In particular, issues with spontaneous defecation and abnormal fecal consistencies are frequently reported, but also deficiencies in fat-soluble vitamins have been linked to orlistat usage (Melia et al., 1996; McDuffie et al., 2002).

H. Lorcaserin

Lorcaserin (Belviq) is a selective serotonin 2C agonist, which has often been referred to as third-generation 5-HT–based anti-obesity pharmacology (Burke and Heisler, 2015). Lorcaserin has been reported to promote satiety to elicit a 3.2% placebo-subtracted body weight loss in overweight and obese adults (Smith et al., 2010; Khera et al., 2016). Mechanism of action presumably involves activation of hypothalamic POMC neurons (Xu et al., 2008; Berglund et al., 2013), without impacting energy expenditure (Martin et al., 2011). Lorcaserin modulates midbrain dopaminergic tone to suppress binge-related food intake (Higgins et al., 2016) and is now being tested for its ability to treat addictive disorders (Higgins and Fletcher, 2015; Shanahan et al., 2017). A series of CNS-related adverse events such as headache, dizziness, fatigue, and nausea have been linked to Lorcaserin treatment. Supporting the notion that the brain plays a seminal role in glycemic control, coadministration of Lorcaserin with metformin or sulfonylureas potentiates the ability of the antidiabetic agents to improve HbA1c and fasting glucose levels in obese subjects with T2D (Moore, 1990). Preclinical studies have explored the prospect of combinatorial targeting of 5-HT2A/C receptors and GLP-1RAs (Anderberg et al., 2017), and it appears that the benefits of Lorcaserin can be increased in combinatorial settings.

I. Rimonabant

Rimonabant antagonizes, by virtue of inverse agonism, the type I cannabinoid receptor (CB1R) to lower body weight by modulating neurons in both homeostatic and hedonic feeding circuits (Cota et al., 2006). Despite pronounced anti-obesity effects with a placebo-subtracted weight loss of ~2.6–6.3 kg (Despres et al., 2005; Van Gaal et al., 2005; Pi-Sunyer et al., 2006), the clinical use of rimonabant was discontinued in 2009 due to serious adverse psychiatric effects (Sam et al., 2011). Notably, this termination was preceded by 2 years of approved medicinal use in Europe. The harmful psychiatric effects have been linked to the antagonizing effect of rimonabant on constitutively active CB1Rs in the ventral tegmental area, and in the amygdala (Meye et al., 2013). Before the clinical development was stopped, rimonabant showed promising effects to improve glycemic control and lessen cardiometabolic risk factors (Despres et al., 2005; Nissen et al., 2008; Van Gaal et al., 2008) Research continues to uncouple the metabolic benefits from the harmful events of CB1R targeting (Simon and Cota, 2017), providing hope that a new generation of safe and efficacious CB1R-based agonists might still be possible.

In summary, since the end of the 19th century, there have been a series of weight-lowering drugs of significant promise that have advanced for the treatment of obesity, and, with the exception of the most recent crop, they have largely failed in measures of chronic safety. Although limited by dose-dependent adverse effects, placebo-subtracted pharmacologically induced weight loss has typically been more than 5%–10%. This level of efficacy pales in comparison with bariatric surgery, but even this magnitude of weight loss can provide a clinically meaningful lessening of obesity-linked comorbidities, most notably T2D and cardiovascular risk factors (Wing et al., 2011). The decrease in hepatic and intra-abdominal fat accumulation, the improvement of β-cell function, as well as enhanced insulin sensitivity in the liver, adipose tissue, and skeletal muscle, are observed with this degree of weight loss (Magkos et al., 2016; Heymsfield and Wadden, 2017). Nevertheless, the proverbial cup is less than half full, and, with the ever-growing burden that obesity and diabetes represent for modern societies, a much more effective and sustainable medicinal solution to complement surgical procedures is desperately needed.

J. Leptin

The adipocyte hormone leptin has like no other appetite-regulating hormone influenced our understanding of how peripheral endocrine signals integrate into the complex central network that controls energy metabolism. Leptin was identified by Jeffrey Friedman in 1994 by positional cloning of the mouse obese (ob) gene (Zhang et al., 1994). Leptin is primarily produced by white adipocytes, from where it is secreted into the general circulation in direct proportion to body fat mass. Leptin acts on the hypothalamic melanocortinergic system to decrease food intake and to increase energy expenditure by stimulating POMC neuronal activity while at the same time silencing neurons that express NPY and AgRP (Schwartz et al., 2000). Beyond its ability to decrease body weight, leptin exerts a remarkable variety of metabolic effects that, among many others, include the regulation of glucose metabolism (Pelleymounter et al., 1995; Hedbacker et al., 2010), stress and anxiety (Haleem, 2014), reproduction (Hebebrand et al., 2007; Müller et al., 2009), inflammation, and hematopoiesis (Fantuzzi and Faggioni, 2000; Zhang and Wang, 2014).

Notably, the discovery of leptin not only identified the adipose tissue as an endocrine organ, it also shaped our understanding of how lipid metabolism can be targeted pharmacologically. Soon after its discovery, leptin was shown to reverse obesity and to improve insulin sensitivity of leptin-deficient ob/ob mice (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995; Hedbacker et al., 2010). These studies created much excitement for the pharmacological use of leptin to treat human obesity. Indeed, exogenous supplementation of leptin corrects obesity in individuals with otherwise low to absent endogenous levels of leptin, such as in ob/ob mice (Campfield et al., 1995; Halaas et al., 1995; Pelleymounter et al., 1995), congenitally leptin-deficient humans (Montague et al., 1997; Farooqi et al., 1999, 2002; Licinio et al., 2004), and individuals with lipodystrophy (Shimomura et al., 1999; Oral et al., 2002; Petersen et al., 2002; Ebihara et al., 2007; Chong et al., 2010). Unfortunately, however, exogenous administration of leptin is largely ineffective to decrease body weight under coditions of common obesity, which is lifestyle/dietary-induced and does not result from a loss-of-function mutation in a single key metabolic gene (Heymsfield et al., 1999; Hukshorn et al., 2000). Such leptin resistance is limiting the use of leptin as a standalone therapy to treat obesity (Hukshorn et al., 2000, 2002; Westerterp-Plantenga et al., 2001). The mechanisms underlying the development of leptin resistance are complex and object of intense scientific investigation. Potential mechanisms include impaired leptin transport across the blood brain barrier (Caro et al., 1996; Banks et al., 1999) or impaired leptin signaling in first- or second-order CNS neurons (El-Haschimi et al., 2000; Wilsey et al., 2003; Münzberg et al., 2004). Dietary fat and sugar seem to be crucial factors leading to leptin resistance, and leptin resistance can occur even before the onset of obesity and hyperleptinemia (Wang et al., 2001; Vasselli, 2008, 2012; Vasselli et al., 2013). Impaired levels of phosphorylated signal transducer and activator of transcription 3 (p-STAT3) can be observed in the arcuate nucleus as early as after 6 days of high-fat diet (HFD) exposure (Münzberg et al., 2004), and even short-term overfeeding of normal weight rats

is sufficient to induce leptin resistance (Wang et al., 2001). In mice, administration of leptin is incapable of preventing the development of obesity when lean mice are switched to a high-sugar HFD at the beginning of the leptin therapy (Müller et al., 2015). In mice, chronic HFD exposure has further been demonstrated to decrease in the number of POMC-positive neurons in the arcuate nucleus (Thaler et al., 2013).

Although the pharmacological use of leptin as a stand-alone therapy to treat obesity is hampered by leptin resistance, leptin is still a valuable constituent for more advanced pharmacological approaches. In line with this notion, adjunct administration of leptin with amylin (Roth et al., 2008), fibroblast growth factor 21 (FGF21), exendin4 (Müller et al., 2012), or a GLP-1/glucagon coagonist (Clemmensen et al., 2014) has all been demonstrated to decrease body weight in diet-induced obese (DIO) rodents beyond what is possible with either compound monotherapy alone. Notably, although the restoration of leptin responsiveness induced by amylin, FGF21, or exendin4 required discontinuation of HFD exposure (Müller et al., 2012; Trevaskis et al., 2016), the GLP-1/glucagon coagonist even improved leptin sensitivity under chronic and persistent exposure of mice to a high-sugar HFD comprising 58% kcal fat (Clemmensen et al., 2014). Also, ER stress has been demonstrated to play a causal role in the development of leptin resistance (Ozcan et al., 2009), and several plant-derived substances, such as celastrol (Liu et al., 2015) and withaferin A (Lee et al., 2016a), have been demonstrated to correct obesity and deranged glycemic control by improving leptin sensitivity in DIO rodents.

IV. From Glucagon-Like Peptide 1 Monoagonism to Multimode Incretin-Based Pharmacology

Historical pharmacotherapies to treat obesity and T2D were often based on the exogenous supplementation of tissue homogenates or extracts obtained and isolated from experimental animals. Many seminal discoveries are based on these crude applications and have collectively primed our understanding of how key endocrine factors promote their biologic action, including how they get transported in and cleared from the circulation. A prominent example is, for example, the observation that pig-derived intestinal mucose homogenate decreases glucosuria in patients with diabetes, suggesting that gastrointestinal hormones regulate pancreatic glucose metabolism (Moore, 1906). Other examples include the use of sheep-derived thyroid extracts to lower body weight (McCone, 1897) or the famous studies showing that administration of pancreatic extracts lowers blood glucose in diabetic dogs and rabbits (Kleiner, 1919; Paulescu, 1921; Banting et al., 1922), seminal observations that subsequently led to the isolation of insulin in 1921 (Banting et al., 1922) and to the identification of glucagon in 1923 (Kimball and Murlin, 1923). Although a series of groundbreaking discoveries are based on the exogenous supplementation of native hormones, such strategy did not translate into a pharmacotherapy capable of satisfactorily decreasing body weight. However, together with constant refinements in biochemial procedures, such as solid-phase peptide synthesis, the knowledge obtained by these historical studies translates nowadays into the ability to synthetically develop pharmaceuticals that differ from the native hormones by improved efficacy and sustained action due to, for example, delayed degradation and clearance from the circulation. Of particular interest emerged biomolecules targeting the receptor for GLP-1.

A. Optimized Glucagon-Like Peptide 1 Monoagonists

A common approach to improve the metabolic benefits of a drug is through refinement of pharmacokinetics. Factors influencing pharmacokinetics typically alter the fate of a drug after its administration, including effects on its liberation from a formulation, followed by its absorption into the general circulation, systemic distribution, metabolic processing, and eventually excretion. In the second half of the last century, a set of complementary chemical and biochemical methods emerged, such as solid-phase peptide synthesis, that provided for the first time the ability to produce and structurally refine macromolecules for therapeutic purposes. Given the seminal importance of insulin, it emerged as a first target for production of the human form of the hormone, followed by chemical analogs that accelerated or prolonged pharmacology following a single injection. Similar technology has been applied to deliver GLP-1 in quantity, quality, and with structural refinement to support therapeutic application as a once-a-day or less frequently administered medicine. The progression of GLP-1 pharmacology to single-molecule polyagonists that possess additional hormone action of differentiated mechanism has been repeatedly reported in recent years to achieve superior metabolic action.

Secreted from intestinal L-cells upon exposure to food, GLP-1 acts at the pancreas to enhance the expression and secretion of insulin (Drucker et al., 1987; Kreymann et al., 1987; Mojsov et al., 1987), and to inhibit the release of glucagon (Schirra et al., 2006). Beyond its role as an insulin secretagogue, GLP-1 agonism can lead to decreases in body weight via central-mediated inhibition of food intake (Sisley et al., 2014a,b; Burmeister et al., 2017). Additionally, it can decrease hepatic glucose output via inhibition of gluconeogenesis (Valverde et al., 1994; Alcantara et al., 1997; Prigeon et al., 2003; Lee et al., 2007), improve insulin sensitivity in skeletal muscle (Idris et al., 2002; Gonzalez et al., 2005), slow gastric emptying (Willms et al., 1996), improve cardiac performance (Sonne et al., 2008; Timmers et al., 2009),

act upon the immune system to decrease inflammation, and stimulate β-cell proliferation and islet mass in rodents (Edvell and Lindstrom, 1999; Wang et al., 1999; Stoffers et al., 2000; Farilla et al., 2002; Rolin et al., 2002; Hui et al., 2003). The numerous beneficial effects of GLP-1 are highlighted in several comprehensive review articles (Drucker, 2006, 2016; Holst, 2007; Sivertsen et al., 2012; Campbell and Drucker, 2013; Sandoval and D'Alessio, 2015).

The ability of GLP-1 receptor agonism to lower body weight and improve glucose metabolism has been well confirmed in numerous preclinical and clinical studies. However, the native hormone demonstrates a very short half-life measured in minutes when administered to humans by i.v. infusion or s.c. injection (Hui et al., 2002). The most rapid inactivation of GLP-1 is mediated by the dipeptidylpeptidase IV (DPP-IV), which cleaves a dipeptide from the N terminus of the native peptide to yield an inactive GLP-$1_{9–36}$ amide or GLP-$1_{9–37}$ (Mentlein et al., 1993; Deacon et al., 1995; Kieffer et al., 1995). Once structurally optimized for improved bioavailability and sustained action, a variety of GLP-1 analogs has advanced to regulatory approval. These medicines include exenatide (Byetta; AstraZeneca, Cambridge, UK), lixisenatide (Lyxumia; Sanofi, Paris, France), liraglutide (Victoza; Novo Nordisk, Copenhagen, Denmark), dulaglutide (Trulicity; Eli Lilly & Co., Indianapolis, IN), and albiglutide (Tanzeum; GlaskoSmithKline, Middlesex, UK). Semaglutide (Novo Nordisk, Copenhagen, Denmark) is a late-stage, long-acting structural refinement related to liraglutide that when coformulated with suitable absorption enhancers is reported to be active in oral application (Gotfredsen et al., 2014; Finan et al., 2015a; Kapitza et al., 2015; Ahren et al., 2017; Blundell et al., 2017).

Exenatide (AstraZeneca, Cambridge, UK) is a 39-amino-acid GLP-1 paralog first identified in the venom of the gila monster (*Heloderma suspectum*). A glycine residue at the second N-terminal amino acid protects the peptide from DPP-IV inactivation, whereas a nine-amino-acid C-terminal extension (CEX) improves the chemical stability by enhancing secondary structure (Neidigh et al., 2001) (Fig. 2). Lixisenatide (Sanofi, Paris, France) is a 44-amino-acid derivate of exenatide, where the proline at residue 39 of exenatide is omitted and the C terminus is extended with six additional lysine residues (Thorkildsen et al., 2003) (Fig. 2). Lixisenatide when compared with exenatide demonstrates a slightly enhanced potency to activate the GLP-1 receptor and a near doubling in half-life of 4 hours (Finan et al., 2015a). Unlike the first two GLP-1 analogs, Liraglutide (Novo Nordisk, Copenhagen, Denmark) is an analog based upon the native GLP-1 sequence, but with the exception that the lysine at residue 28 is replaced with arginine (Fig. 2). Liraglutide is palmitoylated (C16:0) at the side chain of lysine 20 via a γ-glutamic acid spacer. The benefits of this C16 fatty acylation are twofold and pertain to proteolytic stability and time action. Despite being of native sequence, liraglutide is much less susceptible to DPP-IV proteolysis and the fatty acid promotes formation of a self-associated, multimolecular complex at the site of injection to slow diffusion from the site of injection. Furthermore, the palmitic acid facilitates the noncovalent association of the peptide to albumin, resulting in delayed renal clearance and an extended half-life, which in humans is ~12 hours (Agerso et al., 2002). Semaglutide (Novo Nordisk) is a chemically optimized analog of liraglutide with enhanced pharmacological properties. A dicarboxylic-stearic acid (C18:0) is linked to the lysine 20 residue through a γ glutamic acid spacer in a chemical manner that enhances the noncovalent binding to albumin to further decelerate renal clearance (Lau et al., 2015). This enhanced plasma binding results in enhanced pharmacokinetics such that semaglutide manifests a half-life of 160 hours after s.c. injection in humans (Gotfredsen et al., 2014). To support the extended time action, the native alanine at the second residue is substituted with an amino-isobuturic acid (Aib) to further protect against DPP-IV inactivation (Fig. 2). Dulaglutide (Eli Lilly & Co., Indianapolis, IN) is a biosynthetically manufactured, fusion protein comprising two GLP-1 derivatives, each linked to a human Fc fragment of IgG4 to form a dimeric antibody-like protein (Fig. 2). The GLP-1 agonist employed in dulaglutide has a glycine at the second residue to lessen DPP-IV cleavage. Substitution to glutamic acid at position 16 further enhances the secondary structure and potency, whereas glycine at position 30 serves as the junction point to a linking peptide that connects to the IgG Fc fragment (Glaesner et al., 2010). The Fc fragment improves bioavailability of the protein by slowing down its renal clearance. The half-life in humans is reported to be to 90 hours and supports once-weekly administration (Barrington et al., 2011). Albiglutide possesses a 60-amino-acid repeating dimeric agonist where the C terminus of the first GLP-1 agonist is linearly fused to the N terminus of the second (Fig. 2). As employed elsewhere, a glycine at the second position of each agonist minimizes DPP-IV inactivation, and C-terminal covalent coupling of the dimeric agonist to human albumin reduces renal clearance, to provide a half-life reported to be ~120 hours in humans (Bush et al., 2009).

Collectively, these structurally optimized GLP-1 agonists form a class of drugs with potency that varies by more than 10-fold and duration of action suitable for twice-daily to once-weekly s.c. injection. As a class, these GLP-1 analogs provide a sizable and clinically meaningful improvement in glycemic control (Juhl et al., 2002; Chang et al., 2003; Degn et al., 2004; Vilsbøll et al., 2007), and with little to no risk of hypoglycemia (Vilsbøll et al., 2007; Irie et al., 2008; Seino et al., 2008). Although improvement in glucose metabolism has been repeatedly confirmed in

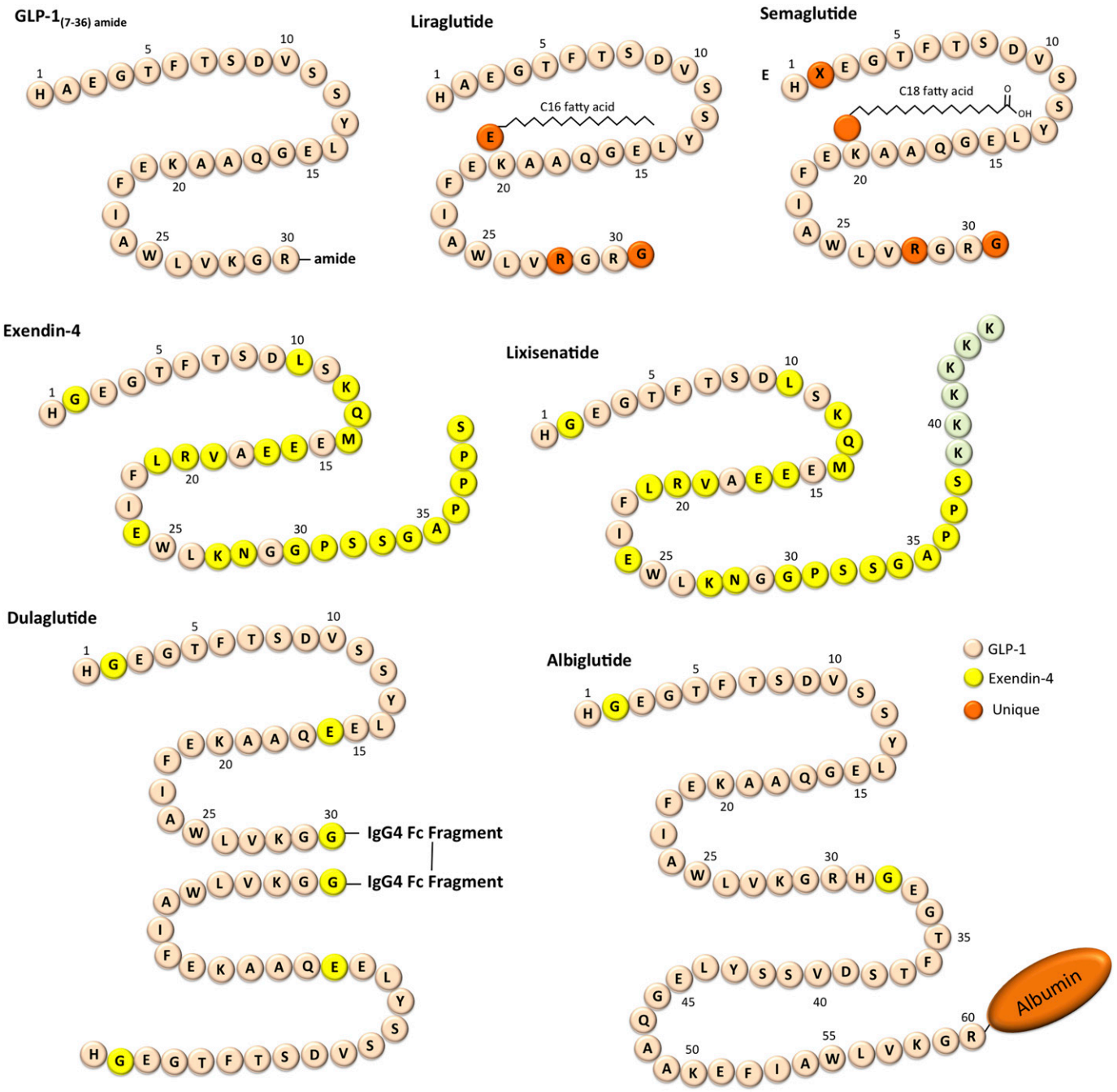

Fig. 2. Schematic of the GLP-1 derivatives approved by the FDA for the treatment of diabetes.

numerous preclinical and clinical studies, higher doses of GLP-1 are required to achieve a meaningful decrease in body weight. Furthermore, GLP-1 agonism confers dose-dependent gastrointestinal adverse effects that serve to limit the therapeutic intensity (Peters, 2013; Bettge et al., 2017). Nevertheless, when used as an adjunct to lifestyle changes, Saxenda (3 mg liraglutide; Novo Nordisk) is approved by the FDA for treatment of obesity. The mean weight loss attributed to Saxenda is 8.4 kg after 56 weeks of treatment, relative to 2.8 kg in placebo-treated controls (Pi-Sunyer et al., 2015), with a finite degree of patients achieving more than 10% absolute body weight reduction.

B. Coadministration of Single Hormones

Most single-hormone pharmacotherapies evaluated for the treatment of obesity show limited efficacy to lower body weight, typically less than 5% and rarely more that 10% relative to placebo-controlled comparison treatment. It seems intuitive to expect that administration of more than one drug, each given at a tolerable dose, might further improve outcomes beyond what is otherwise possible with either hormone alone. Ideally, the

combinatorial approach would synergistically improve metabolism to a greater degree than the sum of the individual therapies alone. Such a polypharmacologic approach has in principle been practiced historically by physicians when prescribing the so-called rainbow pills and, in a most controlled fashion, the use of Qysmia (combination of phentermine and topiramate). More recent preclinical examples include the combination of leptin with the amylin analog named pramlintide (Roth et al., 2008; Trevaskis et al., 2008, 2010; Chan et al., 2009; Turek et al., 2010), or the combination of leptin with exendin-4 or FGF21 (Müller et al., 2012). In all of these studies, the combination of leptin with amylin, FGF21, or exendin-4 improved weight loss synergistically in diet-induced obese rodents when compared with treatment with the respective monotherapies. Other preclinically evaluated GLP-1–based combination therapies include the salmon calcitonin with exendin-4 (Bello et al., 2010), GLP-1 with PYY (Neary et al., 2005), exenatide with CCK (Trevaskis et al., 2015), and liraglutide with an melanocortin 4 receptor agonist (setmelanotide, RM-493) (Clemmensen et al., 2015), which has recently been shown to correct obesity in POMC-deficient humans (Kuhnen et al., 2016). In all of these reports, the combination therapy demonstrated metabolic benefits greater than what can be achieved by the respective hormone monotherapies.

V. Unimolecular Multiagonism: Closing the Gap to Bariatric Surgery

GLP-1 constitutes an appealing target upon which more advanced pharmacological approaches might be built that employ the action of complementary metabolic hormones to a single unimolecular entity. Similar to physical coadministration of single hormones, the basic idea in using a single molecule of dual activity remains common to the belief that simultaneous, complementary biologic mechanisms should enhance metabolic benefits while minimizing adverse effects. One might question whether a single-molecule multiagonist is preferable to administration of multiple independent hormones. The central biologic difference resides in each single hormone possessing a unique pharmacokinetic profile. Consequently, within a comixture, the simultaneously injected hormones differ in rates of absorption, distribution, metabolism, and clearance. Single-molecule polyagonists are ideally suited to function at single target sites where, when possible, they might deliver synergistic or complementary pharmacology. The performance difference that might be obtained is near impossible to predict and needs to be experimentally assessed.

A. Glucagon-Like Peptide 1/Glucagon Coagonism

A provocative approach was the development of a single molecule that recruits the full pharmacology of glucagon along with GLP-1 for the purpose of treating obesity and glucose intolerance (Day et al., 2009) (Fig. 3). At first glance, the combined agonism at the GLP-1 and glucagon receptors seems counterintuitive in providing powerful, but opposing effects on glycemia. Indeed, the most acknowledged metabolic effect of glucagon is its ability to acutely increase glucose levels, given its direct action at the liver to stimulate gluconeogenesis and glycogenolysis (Jiang and Zhang, 2003; Müller et al., 2017). Consistent with this effect is the demonstration that persistent, excessive glucagon action leads to hyperglycemia and eventually T2D. Reports from Roger Unger and associates in 1970 showed that glucose-mediated inhibition of glucagon secretion is impaired in patients with T2D (Müller et al., 1970; Unger et al., 1970). This is a seminal observation that was later confirmed by several independent research groups (Gerich et al., 1976; Felig et al., 1978; Butler and Rizza, 1991; Kelley et al., 1994). Subsequent studies showed that somatostatin-induced inhibition of postprandial glucagon secretion ameliorates hyperglycemia in patients with T2D (Gerich et al., 1974; Dinneen et al., 1995; Shah et al., 2000), and more recently that blocking glucagon action decreases hyperglycemia in a variety of species, including rodents (Mu et al., 2011; Kim et al., 2012b; Okamoto et al., 2017), rabbits (Brand et al., 1996), dogs (Rivera et al., 2007), nonhuman primates (Xiong et al., 2012; Okamoto et al., 2015), and humans (Petersen and Sullivan, 2001; Kelly et al., 2015; van Dongen et al., 2015; Kazda et al., 2016; Kostic et al., 2018). The virtues and limitations of antagonizing glucagon signaling for the treatment of diabetes have recently been highlighted in several review articles (Unger and Cherrington, 2012; Farhy and McCall, 2015; Lee et al., 2016b; Müller et al., 2017), with the implication that excess glucagon action can serve a greater role in the pathology of T2D than impaired insulin action (Unger and Cherrington, 2012). In summary, there is substantial evidence directing inhibition of glucagon action as opposed to enhancing it for the treatment of T2D.

The acute hyperglycemic effect of glucagon argues against its pharmacological use to address excess body weight. However, the glucocentric view of glucagon overshadows the other beneficial effects that it could serve beyond glucose management (Müller et al., 2017). Glucagon acts on the brain to decrease food intake (Salter, 1960; de Castro et al., 1978; Billington et al., 1991); it increases energy expenditure through stimulation of brown fat thermogenesis (Joel, 1966; Kuroshima and Yahata, 1979; Doi and Kuroshima, 1982), inhibits gastric motility (Watanabe et al., 1982; Mochiki et al., 1998; Shibata et al., 2001), decreases fat accumulation via stimulation of lipolysis and inhibition of lipid synthesis (Caren and Corbo, 1960; Salter et al., 1960; Paloyan and Harper, 1961; Amatuzio et al., 1962; De Oya et al., 1971; Eaton, 1973), can improve cardiac performance (Whitehouse and James, 1966;

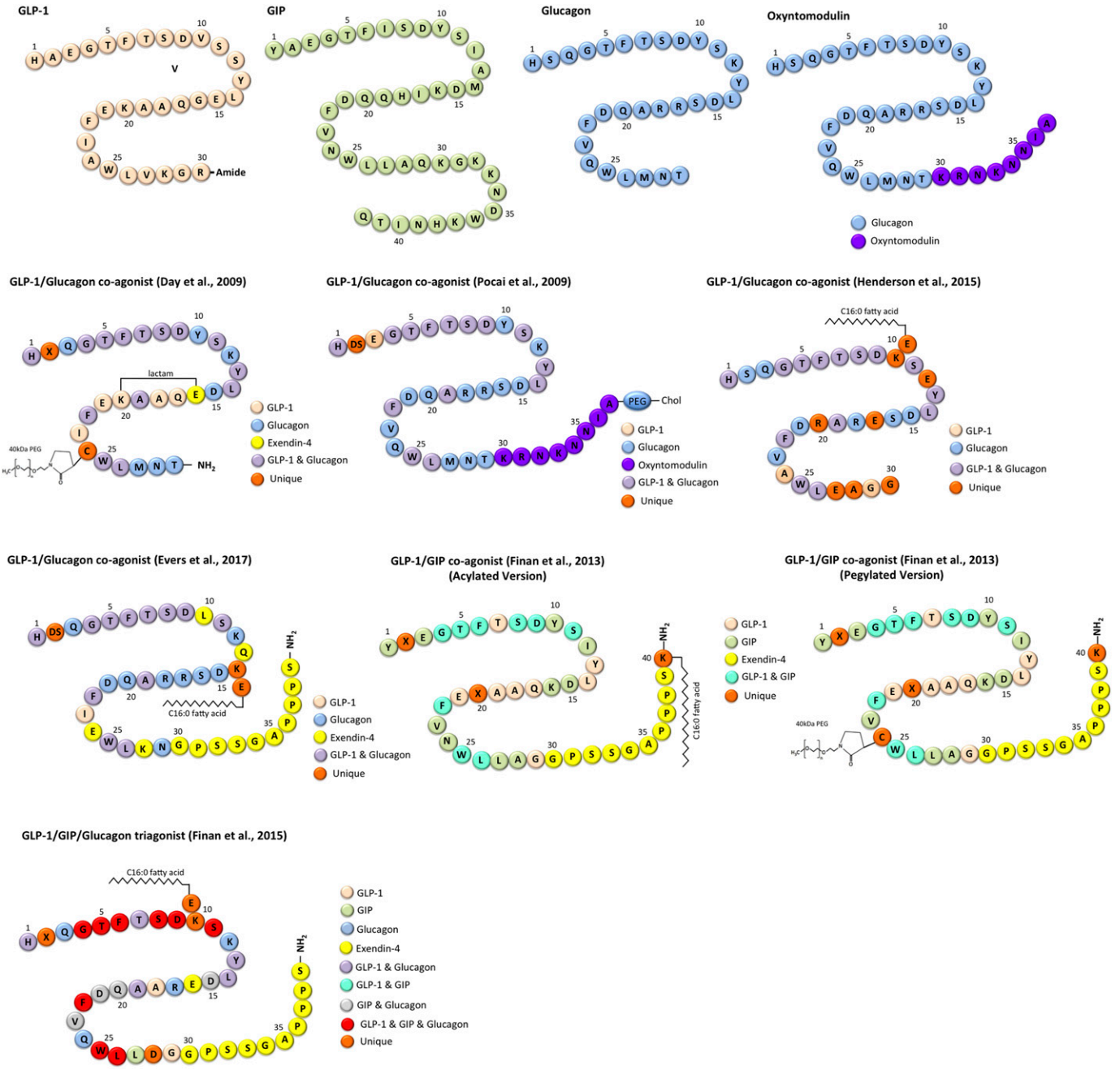

Fig. 3. Schematic of the major peptides of the glucagon family as well as the unimolecular dual and triple agonists targeting the receptors for GLP1, GIP, and glucagon.

Glick et al., 1968; Laraia et al., 1968; Lucchesi, 1968; Katz et al., 1969), and stimulates autophagy (Deter and De Duve, 1967; Arstila and Trump, 1968; Guder et al., 1970; Deter, 1971). Collectively, these nonglycemic effects render glucagon an interesting candidate for pharmacological management of body weight. However, beyond the central dilemma of its diabetogenic liability resides the fact that glucagon is poorly suited as a drug substance, given its short duration of action, poor aqueous solubility, and chemical stability at physiologic pH (Gratzer et al., 1972; Chabenne et al., 2010). The solubility of glucagon in physiologic buffer can be dramatically improved by extension of its sequence at the C terminus with the CEX terminal end of exendin-4 (Li et al., 2007; Chabenne et al., 2010). Chronic s.c. infusion of DIO mice with glucagon-CEX in physiologic buffer improved body weight and glycemic control with equal efficacy when compared with equimolar administration of exendin-4 (Müller et al., 2017). This observation made in 2006 was seminal to the realization that glucagon could be used to improve body weight and metabolic control, but also emphasized that it possessed a narrow therapeutic index. Consequently, in search of a means to enhance its efficacy and broaden its safety

emerged the development of unimolecular peptides with full, balanced activity at the receptors for each of these peptide hormones.

Collaborative academic research conducted in the DiMarchi and Tschöp laboratories led to the discovery of a set of peptides of varying degrees of GLP-1 and glucagon coagonism (Day et al., 2009). Conceptually these molecules were based on the assumption that the beneficial glycemic effects of GLP-1 would restrain the hyperglycemic potential of glucagon, whereas the anorectic effect of central GLP-1 agonism would complement glucagon's own anorectic, lipolytic, and thermogenic properties to maximize body weight loss. Fortuitously, these two hormones have high sequence homology, which facilitated the search for full potency, balanced coagonists. The fact that selective recognition of glucagon and GLP-1 by their cognate receptors could be modulated through the exchange of specific amino acids at the peptide terminal ends was something first reported a decade earlier (Hjorth et al., 1994).

The initial GLP-1/glucagon coagonists studied by DiMarchi, Tschöp, and colleagues were glucagon-based in which amino acids 17, 18, 20, 21, and 23 were substituted to the respective GLP-1 residues (Day et al., 2009). The alanine at position 2 of the peptide was substituted with Aib to protect the molecule from DPP-IV inactivation, and a lactam bridge was introduced between glutamic acid 16 and lysine 20 to covalently stabilize the secondary structure to enhance glucagon receptor (GcgR) potency (Fig. 3). To support in vivo studies, a 40-kDa polyethylene glycol (PEG) was site-specifically attached to the side of cysteine 24 to prolong in vivo action. The resulting peptide maintained nearly balanced coagonism at both receptors. It was highly soluble in physiologic buffer (>25 mg/ml) and chemically stable for more than 1 week when incubated in plasma. Despite being slightly less potent than the native hormones, the potency to activate both receptors was still subnanomolar, rendering it a suitable candidate for initial preclinical testing. Once-weekly administration for 4 weeks in diet-induced obese mice (70 nmol kg^{-1}) of this nearly balanced dual agonist was sufficient to correct diet-induced obesity and hepatic steatosis, while improving glucose tolerance and cholesterol metabolism. Notably, the dual agonist showed much superior metabolic action relative to a structurally similar (no lactam bridge) analog with otherwise comparable pharmacokinetics and GLP-1 potency, but reduced 10-fold in glucagon activity. Weight loss induced by the dual agonist was predominantly due to decreased body fat mass and was accompanied by lower food intake and elevated energy expenditure. The solid increase in energy expenditure was in line with published reports on glucagon agonism (Joel, 1966; Kuroshima and Yahata, 1979; Doi and Kuroshima, 1982) and was not observed with a GLP-1–selective molecule without GcgR agonism.

These observations are consistent with GLP-1's anorectic effect integrated with glucagon's capacity to increase thermogenesis. To a lesser magnitude relative to what was observed in obese wild-type mice, the dual agonist also lowered body weight in mice lacking the GLP-1 receptor, thus corroborating that GcgR agonism is a valuable constituent to the combined pharmacology (Day et al., 2009). Of appreciable note, GLP-1/glucagon dual agonism also effectively lowers body weight and improves glycemia in nonhuman primates (Tschop et al., 2016), an important observation later also confirmed with another GLP-1/glucagon dual agonist (Henderson et al., 2016). Another interesting and unexpected finding was the ability of such a dual agonist to improve leptin sensitivity of DIO mice, despite continued chronic exposure of the mice to a high-sugar HFD (Clemmensen et al., 2014). In this dietary paradigm, physical combinations of leptin and exendin-4 or FGF21 had failed to similarly improve leptin sensitivity (Müller et al., 2012), implying a synergistic pharmacology inherent to a single-molecule coagonism.

The initial 2009 coagonist report was received with healthy scientific skepticism, but the independent confirmation at other research sites and the translation from obese rodents to nonhuman primates have supported the advancement of the concept to human studies (Day et al., 2009, 2012; Pocai et al., 2009; Henderson et al., 2016). Separately, low-dose coinfusion of GLP-1 and glucagon has been demonstrated to decrease food intake (Cegla et al., 2014) and to increase energy expenditure in humans (Tan et al., 2013). Several unimoleculer GLP-1/glucagon dual agonists that vary in the relative ratio of the two activities are currently in clinical evaluation for the treatment of obesity and diabetes (Finan et al., 2015a; Brandt et al., 2018).

It is worth noting that, independent of the directed synthesis of glucagon–GLP-1 coagonists, work with oxyntomodulin was occurring. This peptide is an endogenous precursor to glucagon of much lower inherent potency, and less balanced in GLP-1 agonism. In a chemical sense, oxyntomodulin constitutes an eight-amino-acid C-terminal extension to glucagon (Fig. 3). It is cosecreted with GLP-1 from intestinal L-cells. As demonstrated by in vitro studies and in isolated tissue samples, oxyntomodulin (OXM) is able to bind and activate both GLP-1R and GcgR, but with a 10- to 100-fold lower affinity relative to native GLP-1 and glucagon (Bataille et al., 1982; Baldissera et al., 1988; Gros et al., 1995; Schepp et al., 1996; Jorgensen et al., 2007). OXM reduces food intake and lowers body weight in rodents (Dakin et al., 2001, 2002, 2004) and humans (Wynne et al., 2005, 2006). Notably, OXM inhibition of food intake is abrogated in mice lacking GLP-1R (Baggio et al., 2004; Sowden et al., 2007) but is preserved in mice lacking GcgR (Baggio et al., 2004), suggesting that OXM inhibition of food intake is mediated via only by the GLP-1 receptor. However, side-by-side comparison of OXM to a molecule in which

the GcgR activity of OXM had been completely removed showed a superior ability of OXM to lower body weight and fat mass. This suggests that the glucagon receptor activity of OXM is a participant in pharmacologically induced weight loss (Kosinski et al., 2012).

Just a few days after the Day et al. (2009) GLP-1/glucagon coagonist publication, the research group at Merck reported the development of an OXM-based peptide with glucagon and GLP-1 agonism (Pocai et al., 2009). Relative to native OXM, this DualAG peptide showed improved pharmacokinetics and comparable potency to activate GLP-1R and GcgR. A 14-day treatment of DIO mice decreased body weight and improved glucose metabolism. Notably, improvement of systemic metabolism by this DualAG peptide (Fig. 3) was abolished in mice lacking either the GLP-1R or GcgR. This confirmed the complementary activity of this molecule at both receptors when used at pharmacological levels (Pocai et al., 2009). From this point, the chemical optimization of OXM has been guided by the higher inherent potency in glucagon-based analogs, without any apparent need for the cationic C-terminal extension found in nature. To what degree OXM functions endogenously as a physiologic coagonist to modulate glucose and body weight remains an unanswered question, but, given its low inherent bioactivity coupled with the low plasma concentrations, its primary function appears to be the historically viewed biosynthetic precursor to glucagon. Following their introduction in 2009 (Day et al., 2009), several dual agonists targeting the receptors for GLP-1 and glucagon have been developed (Fig. 3) and their efficacy translates from obese rodents to nonhuman primates and humans (Day et al., 2009; Pocai et al., 2009; Henderson et al., 2016; Tschop et al., 2016; Evers et al., 2017).

B. Glucagon-Like Peptide 1/Amylin Coagonism

Islet amyloid polypeptide is a 37-amino-acid peptide produced and cosecreted with insulin from the pancreatic β-cells, which is more commonly named amylin. Like insulin, circulating levels of amylin are positively correlated to levels of blood glucose and consequently low in hypoglycemia (Mitsukawa et al., 1990), largely absent in individuals with type 1 diabetes (Clark et al., 1990; Hartter et al., 1990; Ogawa et al., 1990; Bretherton-Watt et al., 1991; Young, 2005), and, depending on the progression of the disease, elevated or decreased in individuals with T2D (Cooper et al., 1987, 1988; Westermark et al., 1987; Johnson et al., 1989; Enoki et al., 1992). It is cosecreted with insulin, and, upon glucose stimulation, amylin returns signal back to the β-cells to suppress insulin secretion, under basal conditions (Silvestre et al., 1990) and after stimulation with either glucose (Ohsawa et al., 1989; Silvestre et al., 1990) or arginine (Inoue et al., 1993). Beyond its ability to regulate the release of insulin, amylin decreases gastric acid secretion, delays gastric emptying, and inhibits glucagon secretion (Woods et al., 2006; Lutz, 2010a,b). Upon central or peripheral administration, amylin dose-dependently decreases body weight via inhibition of food intake (Chance et al., 1991; Lutz et al., 1994; Lutz, 2010b). Given its systemic metabolic effects, as expected blocking amylin signaling either through administration of an amylin receptor antagonist (Rushing et al., 2001) or through genetic ablation of amylin (Lutz, 2005) increases food intake and body weight in rodents. Amylin's anorectic action seems to be mediated in the area postrema because selective administration of amylin to this region decreases food intake, whereas lesion of the area postrema blocks amylin's anorectic effect (Lutz et al., 1998; Riediger et al., 2001, 2004; Becskei et al., 2007; Mack et al., 2010). Pramlintide (Amylin Pharmaceuticals, San Diego, CA) is a synthetic amylin analog in which the human amylin sequence has been modified to include prolines at residues 25, 28, and 29, as occurs in rat sequence. It is a registered medicine for the treatment of diabetes, and treatment with insulin has proven to improve glucose metabolism in individuals with type 1 diabetes (Thompson et al., 1997b; Weinzimer et al., 2012; Herrmann et al., 2013) and as an independent agent in T2D (Thompson et al., 1997a; Riddle et al., 2007). The mechanism of action includes a slowing of gastric motility (Kong et al., 1997, 1998) and inhibition of glucagon secretion (Nyholm et al., 1999; Levetan et al., 2003).

Calcitonin and amylin biochemically signal through a common family of G protein–coupled receptor family B receptors. Intramuscular coadministration of salmon calcitonin with exendin-4 synergistically lowers food intake in nonhuman primates (Bello et al., 2010), an observation that inspired the development of unimolecular peptide hybrids (phybrids) targeting the receptors for GLP-1 and amylin (Sun et al., 2013; Trevaskis et al., 2013). Two of these phybrids are constituted by a C-terminally truncated exenatide, which at its C terminus is covalently linked to the N terminus of an amylin analog (davalintide) through either a repeating β-Ala-β-Ala dipeptide, or through triple-glycine linear repeat (Trevaskis et al., 2013). As assessed in rodent models of obesity, weight loss induced by these phybrids is greater than what is observed with each receptor monoagonist alone, but is similar to what is achieved by a physical comixture of the single hormones (Trevaskis et al., 2013). Another GLP-1/amylin phybrid uses a full-length exenatide sequence that is linked to davalintide via an intervening 40-kDa PEG (Sun et al., 2013). In rodent models of obesity, this phybrid dose-dependently improved glucose handling and body weight with superior in vitro and in vivo potency relative to a side-chain, PEGylated phybrid (Sun et al., 2013).

C. Glucagon-Like Peptide 1/Glucose-Dependent Insulinotropic Polypeptide Coagonism

Another unexpected controversial approach was the development of a molecule with dual agonism at the

receptors for GLP-1 and the GIP, with the primary indication treatment of glucose intolerance (Finan et al., 2013) (Fig. 4). The 42-amino-acid peptide GIP is produced by K-cells in the duodenum and jejunum and is released into the general circulation upon stimulation by dietary nutrients, and especially lipids (Takeda et al., 1987; Lardinois et al., 1988; Inagaki et al., 1989). First isolated from porcine intestinal extracts, GIP was initially shown to inhibit gastric acid secretion in dogs, leading to the characterization as a gastric inhibitory polypeptide (Brown and Pederson, 1970; Brown, 1971). Work by Dupre et al. (1973) then demonstrated that i.v. administered GIP increases plasma levels of insulin in humans, and thus served to identify GIP as the first incretin hormone. Subsequently, GIP was shown to directly act on the pancreas to enhance glucose-stimulated insulin secretion (Dupre et al., 1973; Taminato et al., 1977; Adrian et al., 1978), and with it the reclassification of the hormone as a glucose-dependent insulinotropic polypeptide. Of appreciable note, beyond its ability to stimulate the release of insulin under conditions of hyperglycemia, GIP also stimulates the release of glucagon under conditions of hypoglycemia and thus represents a bifunctional hormone capable of buffering against the extremes in glucose excursion highs and lows (Pederson and Brown, 1978; Meier et al., 2003; Christensen et al., 2011, 2014).

Although the insulinotropic action of GIP renders this peptide an attractive pharmacological target, GIP agonism has long been regarded as a causal factor implicated in the development of obesity and insulin resistance (Finan et al., 2016b). The view of GIP as a putative obesogenic factor was supported by reports that circulating levels of GIP are positively correlated with body weight, and are typically elevated in genetically- and diet-induced obese mice (Flatt et al., 1983; Bailey et al., 1986; Miyawaki et al., 2002) and obese humans (Creutzfeldt et al., 1978; Salera et al., 1982; Calanna et al., 2013). The obesogenic nature of GIP is seemingly also supported by in vitro studies showing that GIP has lipogenic and adipogenic effects on adipocytes through mechanisms that include stimulation of adipogenesis (Eckel et al., 1979), inhibition of lipolysis (Gogebakan et al., 2012), and stimulation of de novo lipogenesis (Oben et al., 1991). Additionally, it stimulates triglyceride release from chylomicrons (Wasada et al., 1981; Ebert et al., 1991), adipocyte glucose and fatty acid uptake (Beck and Max, 1986; Hauner et al., 1988), and adipocyte lipoprotein lipase enzyme activity (Eckel et al., 1979; Knapper et al., 1993; Kim et al., 2007). Consistent with these biochemical properties, a series of studies embellished the belief of GIP as a lipogenic hormone as blocking its action either through targeted ablation of GIP-producing K-cells (Althage et al., 2008), genetic ablation of the GIP receptor (Miyawaki et al., 2002), or through immunoneutralization (Montgomery et al., 2010), diminished body weight gain, and improved glucose metabolism in mice chronically exposed to a HFD. Notably, selective genetic ablation of the GIP receptor in β-cells decreases postprandial insulin levels in chow-fed mice, but does not protect them from obesity when exposed to high-fat feeding (Campbell et al., 2016). These data might indicate that the anti-obesogenic effect in inhibition of GIP action might not necessarily reside in the lack of GIP action on adipose tissue, but rather a consequence of diminished insulinotropic action resulting in reduced insulin adipose action (Finan et al., 2016b). Notably, in contrast to a series of historic studies testifying to GIP as an obesogenic hormone, mice overexpressing GIP show improved β-cell function and improved glycemic control and are resistant to diet-induced obesity (Kim et al., 2012a). Furthermore, chronic GIP receptor (GIPR) agonism was recently shown to improve glucose metabolism in DIO mice, without detrimental effects on body weight (Martin et al., 2013). The importance of functional GIP signaling can clearly be seen in transgenic pigs expressing a dominant-negative (dn) GIP pancreatic receptor (Renner et al., 2010). These GIPR(dn) pigs show impaired glucose tolerance due to delayed insulin secretion, impaired insulinotropic action of GIP, up to 60% reduced β-cell proliferation, and reduced islet mass of up to 58% at the age of 1 year (Renner et al., 2010), all hallmarks of the progression to T2D.

The rationale in combining the pharmacology of GIP and GLP-1 to a single molecule resides in the well-established insulinotropic action of both peptides, which they achieve in part by distinct mechanisms (Müller et al., 2017). At minimum, GIP agonism would augment GLP-1's glycemic effect, whereas the anorectic effect of GLP-1 could buffer against the purported obesogenic liability of GIP. Several studies have studied coinfusion of GLP-1 and GIP agonists. In rodents, combined agonism at these two receptors synergistically decreases body weight in DIO rodents (Finan et al., 2013) and improves glucose control relative to monotherapies. There was no additive effect observed on body weight in obese leptin-deficient ob/ob mice (Gault et al., 2011). In humans, coinfusion of GLP-1 and GIP analogs additively increases the insulinotropic action relative to infusion with either agonist alone (Nauck et al., 1993a). Importantly, patients with T2D appear unresponsive to the insulinotropic action of the hormone (Nauck et al., 1993b; Vilsbøll et al., 2002), and there are also reports indicating that GIP agonism does not potentiate the glycemic benefits of GLP-1 agonism in hyperglycemic patients (Mentis et al., 2011). Nevertheless, two unimolecular GLP-1/GIP coagonists were developed and preclinically tested by DiMarchi, Tschöp, and colleagues (Finan et al., 2013). The conceptual design of these GLP-1/GIP coagonists was similar to that of the previously reported GLP-1/glucagon coagonists, where a single peptide of mixed

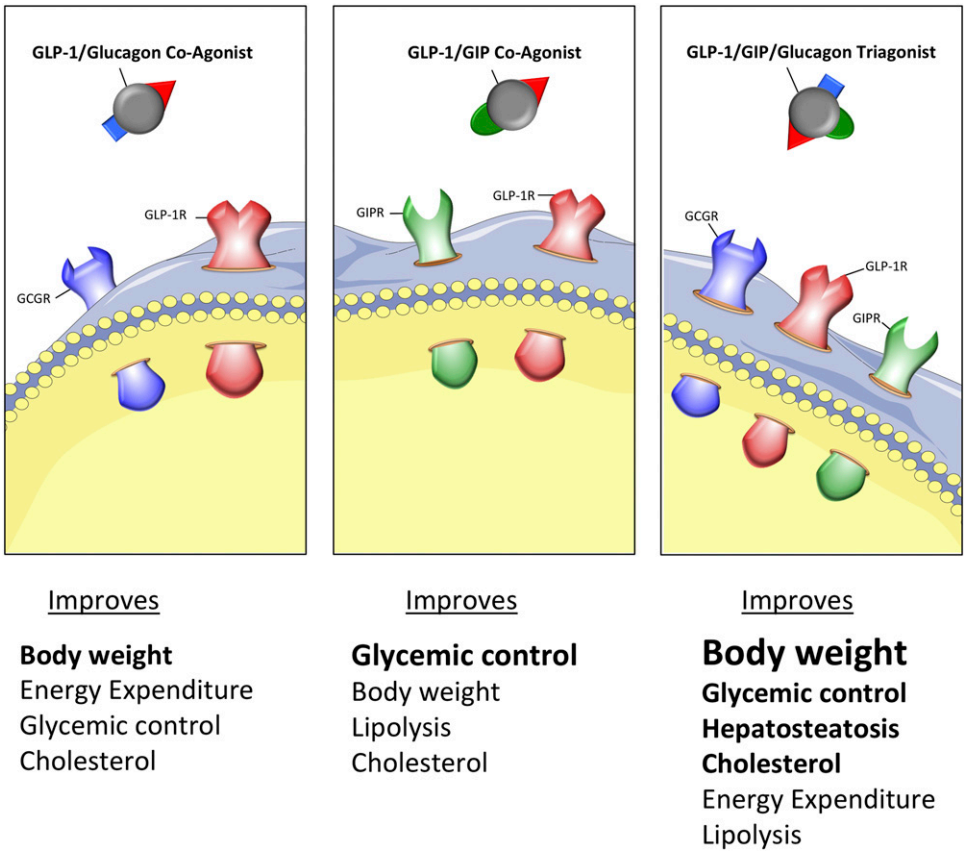

Fig. 4. Schematic on the principle and metabolic action of GLP-1/glucagon, GLP-1/GIP, and GLP-1/GIP/glucagon.

sequence was identified that displayed full and balanced potency at both receptors. GIP residues were introduced in the middle and C-terminal part of the peptide, whereas certain modifications that enhanced GcgR activity were removed (Finan et al., 2013). The C terminus of the peptide ended with the nine-amino-acid extension (CEX) found in exendin-4, and an Aib at position 2 to protect against DPP-IV inactivation (Fig. 3). To extend in vivo time action, the dual-agonist peptides were either site-specifically modified with a 40-kDa PEG at Cys-24, or directly fatty-acylated at the Lys40 with palmitic acid. Both peptides possess balanced receptor activities of slightly enhanced (fatty-acylated) or slightly diminished potency (PEG) relative to the native hormones. In preclinical evaluation in diet-induced obese and diabetic, leptin-deficient db/db mice, each of these coagonists demonstrated superior weight-lowering and enhanced improvement of insulin resistance and glucose control relative to pharmacokinetically-matched, best-in-class GLP-1 monoagonists (Finan et al., 2013). Glycemic improvements as expected were achieved through enhanced insulinotropic efficacy, and this notably translated from rodent models of obesity to nonhuman primates and humans (Finan et al., 2013; Portron et al., 2017; Schmitt et al., 2017). Notably, weight loss induced by this twincretin peptide was mediated by inhibition of food intake with no effects on energy expenditure (Finan et al., 2013).

D. Glucagon-Like Peptide 1/Glucagon/Glucose-Dependent Insulinotropic Polypeptide Triagonism

GLP-1, GIP, and glucagon are peptides of similar size with a high degree of homology in sequence that biochemically signal through homologous G protein–coupled surface receptors. The common structural features render them attractive candidates for purposefully achieving full agonism at all three receptors, although there is no precedent for doing so and nature has designed them to be specific for their individual receptors. Based on the demonstrated metabolic benefits of the already published GLP-1/glucagon and GLP-1/GIP coagonists (Day et al., 2009; Finan et al., 2013), it was envisioned that simultaneous balanced agonism at all three target receptors could provide unrivaled metabolic benefits, beyond what had already been achieved with each dual agonist relative to monotherapy (Fig. 4).

The strategic design was based on GLP-1 anorectic effect synergizing with glucagon's lipolytic and thermogenic properties to decrease body weight, whereas the combined insulinotropic action of GLP-1 and GIP would doubly restrain glucagon's hyperglycemic liability to potentially allow more aggressive use.

Relative to GLP-1/GIP coagonists, which are reported to not increase energy expenditure, the glucagon component of the triagonist contributed energy expenditure mechanisms, thus allowing greater body weight–lowering potency. Conversely, the relative potency ratio of the glucagon component in GLP-1/glucagon coagonists will ultimately have to be reduced to favor of GLP-1 potency to avoid any remnant of a glucagon-driven diabetogenic effect. With GIP activity integrated into a triagonist, an independent mechanism is dialed into the molecule that further buffers against glucagon-induced hyperglycemia. This ultimately permits a mixed agonist profile in which the relative potency at each receptor can be balanced such that glucagon activity can be kept at a maximum. Lastly, the GIP component contributes additional improvements in hormonal sensitivity, notably insulin sensitivity, thus reducing basal insulin levels. In doing so, the GIP component lessens the obesogenic drive from hyperinsulinemia.

The triple agonist was based upon the structure–activity studies that had associated with the achieving balanced GLP-1/glucagon and GLP-1/GIP coagonists (Day et al., 2009; Finan et al., 2013). An Aib at position 2 protected the molecule from DPP-IV inactivation, and the lysine at residue 10 was fatty-acylated with a palmitic acid through a γ glutamic acid linker (Fig. 3). Similar to liraglutide, the lipidation promotes noncovalent binding to albumin to slow renal clearance and extend duration of in vivo action. The Aib at position 2 inhibits DPPIV degradation, but also decreases potency at the glucagon receptor. As such, Glu16, Arg17, Gln20, Leu27, and Asp28 were introduced to restore balanced glucagon bioactivity at this receptor. The peptide contains the C-terminal exendin-4 extension sequence (CEX) and displays balanced and full agonism at all three receptors, with 10-fold superior potency relative to the native hormones (Finan et al., 2015b). As shown in a variety of genetically and diet-induced obese and glucose-intolerant mouse models, the triagonist potently decreased body weight through inhibition of food intake and stimulation of energy expenditure. It improved insulin sensitivity, glucose, and lipid metabolism, lowering plasma cholesterol and reversing hepatic steatosis (Finan et al., 2015b). Of appreciable note, the triagonist lowered food consumption and improved glycemic control with similar efficacy relative to the GLP-1/GIP coagonist, yet with greater weight loss given the lipolytic and thermogenic actions attributed to agonism at the glucagon receptor. Validation of the molecular source of the efficacy was established in specific loss-of-function mouse models, including GLP-1R$^{-/-}$, GcgR$^{-/-}$, and GIPR$^{-/-}$ mice (Finan et al., 2015b). Importantly, the triagonist lowered body weight with equal efficacy in obese male and female mice (Jall et al., 2017) and translated from obese rodent models to nonhuman primates (Tschop et al., 2016).

Building upon the initial triple-agonist report, a protein with activity at all these three receptors was reported. In this molecule, coding sequences for GLP-1, GIP, and glucagon in various combinations were genetically fused to the N terminus of the heavy or light chain of a registered monoclonal antibody (Synagis) that is widely used to treat respiratory-syncytial-virus infections (Wang et al., 2016). Relative to the native hormone, this triple agonist displayed comparable in vitro potency at each of the three receptors and half-life that was extended by more than 100-fold. This triple-agonist, antibody-based protein synergistically improved body weight and glucose metabolism in DIO rodents (Wang et al., 2016).

VI. Peptide-Mediated Delivery of Nuclear Hormones

Nuclear hormones are powerful medicinal agents of exceptionally high potency and pleiotropic action profile and have proven particularly useful in treatment of endocrine disorders such as the metabolic syndrome. However, their broad systemic action often results in unwanted adverse effects that restrict the use of these powerful hormones. Many nuclear hormones have multiple receptor isoforms that are believed to serve different physiologic functions and have different tissue distribution patterns. Traditional small-molecule medicinal chemistry has been employed to engineer ligands that selectively function at a specific receptor isoform. However, this approach has failed in most instances to generate drug candidates that demonstrate pharmacology sufficiently selective for chronic human use. An alternative strategy employs macromolecules such as peptides or proteins to direct the biodistribution of the nuclear hormones. The tissue preference is dependent upon the receptor distribution of the targeting ligand, and in theory can be finely tuned by appropriate selection. An additional benefit of this approach is the potential to integrate the inherent pharmacology of the targeting peptide to complement that of the nuclear hormone. In analogy, antibody-based chemotherapy has been employing a similar targeting strategy devoid of the supplemental pharmacology of the targeting ligand to enhance the narrow therapeutic window of high-potency cytotoxic agents. In endocrine applications, the magnitude of therapeutic improvement is considerably less as many of these nuclear hormones, such as estrogens, androgens, and thyroid hormones, are currently used, but with careful dose management.

This strategy employs a covalent linkage of a nuclear hormone to a peptide, preferably through linker that would metabolize to release the nuclear hormone only within the targeted cell (Fig. 5). In principle, such a design restricts the otherwise passive transport of a nuclear hormone through virtually all cell membranes

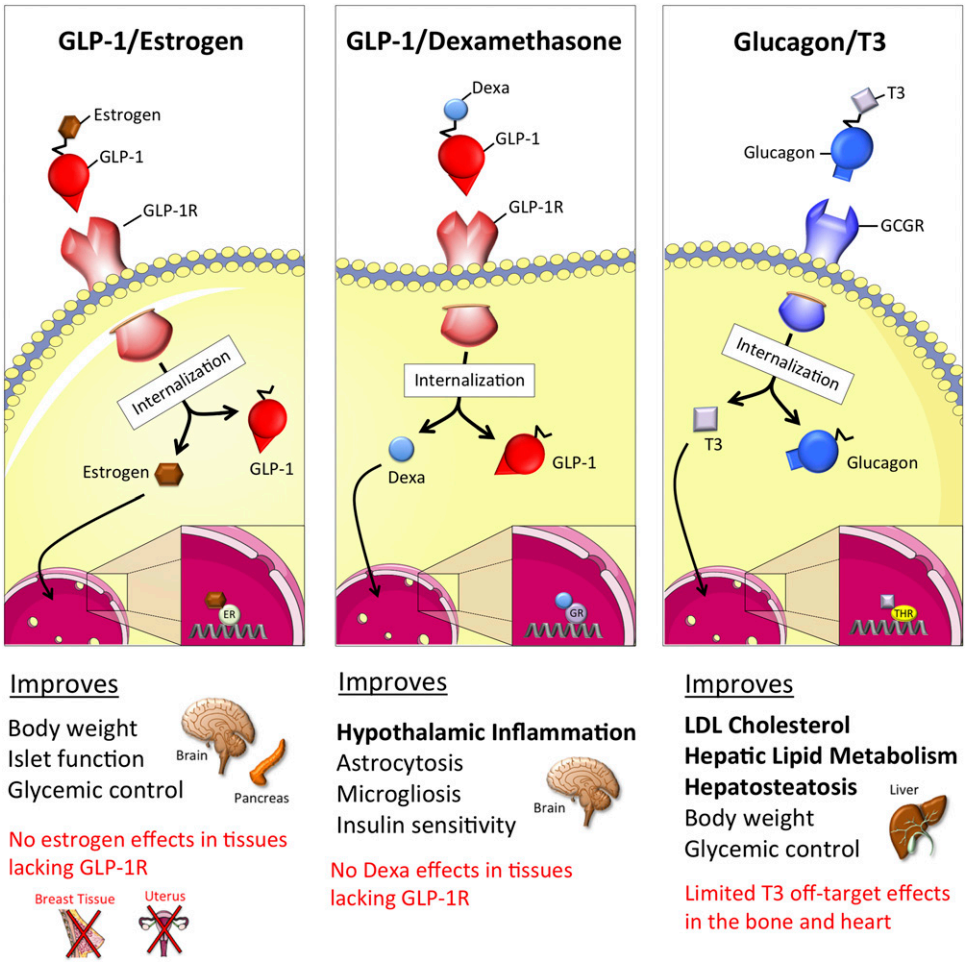

Fig. 5. Schematic on the principle of peptide-mediated nuclear hormone delivery and metabolic effects of GLP-1/estrogen, GLP-1/dexamethasone, and glucagon/T3.

or restricts the presentation of the nuclear hormone to endogenous cellular transporters, and thus lessens the adverse effects in undesirable tissues. However, in cell types that possess the peptide receptor, activation of the cell surface receptor should lead to internalization of the ligand–nuclear hormone receptor complex. In essence, the peptide receptor serves as a gateway into the cell. Upon internalization, biologic processing of a suitably designed linker would release the nuclear hormone and allow it to activate its intracellular receptor. In certain instances, it could be beneficial to release a chemically modified nuclear hormone such that cellular efflux is prevented, or alternatively use an isoform-selective nuclear hormone analog to achieve selectivity otherwise not possible in targeting the native nuclear hormone. As long as there is sufficient nuclear hormone released intracellularly and that it retains sufficient potency at its receptor, complementary biologic effects should result.

There are many inherent concerns and limitations to the approach that require optimization for each application. Potency alignment is one of the uncertainties inherent to all combinatorial therapies, and, unquestionably, physical mixtures are more easily titrated to determine the optimal balance. Additionally, because the peptide works to concentrate the nuclear hormone at target cells, it is more challenging than coagonism at two cell surface family B G protein–coupled receptors where the ligand typically loses all receptor potency with biometabolism of the peptide. Furthermore, whether this approach can be used with peptide ligands that are designed to target more than one target tissue remains to be determined, as it would potentially broaden the pharmacology to increase efficacy, but also toxicity.

Expanding the peptide and nuclear hormone pairings to target additional metabolic pathways or treat other diseases is an exciting proposition. However, the hormone selection requires a judicial choice as not all nuclear hormones are compatible, and many surface-acting agonists are insufficiently selective in sites of action, such as insulin or insulin-like growth factor-1. One inherent attraction to peptide and nuclear hormone pairing is that both entities are typically of high inherent potency, and most peptides have fairly high tissue selectivity. In this regard, peptide receptor antagonists

and intracellularly acting, small-molecule inhibitors are less attractive candidates. Peptide antagonists relative to agonists exhibit slower and more restricted internalization of bound-receptor complexes. Furthermore, not all peptide receptors are internalized. Lastly, most small-molecule inhibitors require appreciably high concentrations, which present challenges to transport a sufficient quantity of material and would potentially require a reduction in peptide potency to achieve balanced compatibility. The latter of these can present biophysical and synthetic obstacles to realizing successful pharmacology.

A. Glucagon-Like Peptide 1–Mediated Delivery of Estrogen

Estrogens have substantial regulatory actions to influence metabolic control. Appreciable experimental evidence exists that demonstrate that many female rodent models are protected from dietary- and genetically-induced metabolic dysfunction (Mauvais-Jarvis et al., 2017a). The most often cited evidence of estrogen's regenerative benefits derives from the many studies of estrogen replacement therapy in postmenopausal women improving multiple cardiometabolic parameters (Mauvais-Jarvis et al., 2017b). Specifically, estrogens have been reported to have protective, anabolic, and insulinotropic effects in rodent pancreatic islets that serve to improve glycemic control (Mauvais-Jarvis, 2016). Separately, estrogens have potent anorectic effects that originate in distinct brain regions that promote body weight loss after exogenous administration (Gao et al., 2007). Interestingly, these pharmacological actions of estrogen coincide with many biologic aspects in GLP-1 receptor agonism.

The rationale for building single-molecule conjugates of GLP-1 and estrogen arose from the overlapping, yet presumed nonredundant mechanisms of GLP-1 and estrogen in metabolic control. By leveraging the preferential sites of GLP-1 action, most notably the endocrine pancreas and hypothalamic feeding circuits, it was hypothesized that GLP-1 could selectively deliver estrogen to these tissues. Restricting the site of estrogen action would in theory limit the reproductive endocrine toxicity and oncogenic liability of unopposed, systemic estrogen. Whether this could provide a meaningful enhancement in GLP-1 pharmacology and an improved therapeutic window for chronic estrogen use is difficult to know without experimentation. Consequently, a series of GLP-1 and estradiol (E2) conjugates were generated using a DPP-IV–resistant GLP-1 analog with stable, ether-based linker between the peptide and E2 (Finan et al., 2012) (Fig. 5). In addition, a series of meta-stable linkers were explored that were rationally designed to be selectively sensitive to intracellular degradation, yet stable in plasma by taking advantage of the different physiologic conditions inside of a cell. Numerous control compounds included the following: a peptide with selective chemical knockout of the GLP-1 potency through the use of point mutations or complete d-amino acid substitution of the sequence, a conjugate with a labile phenolic ester-based linker that rapidly decomposes in circulation after administration to release systemic E2, a peptide conjugated to lithocholic acid as a pharmacokinetic control because the bile salt has similar lipophilicity as E2, and finally a GIP-E2 conjugate to impart differential tissue delivery of E2.

Administration of a stable GLP-1/E2 conjugate dose-dependently decreased body weight and improved glycemic control in various rodent models of the metabolic syndrome, including diet-induced obese mice and db/db mice (Finan et al., 2012). The weight-lowering benefits were the result of collective effects to suppress food intake, and the GLP-1/E2 conjugate showed greater potency relative to the GLP-1 analog or E2 control to reduce food intake and lower body weight. Subsequent studies have reported effects of the GLP-1/E2 conjugate on feeding behavior and reward (Cao et al., 2014; Vogel et al., 2016). These enhanced metabolic benefits were noticeably absent following treatment with chemically-inactive GLP-1 conjugates, or conjugates with a labile linkage to estrogen, or stable conjugates to bile acids, and the stable E2 conjugate to GIP. The absence of amplification with these control peptides demonstrates the targeting in the combined pharmacodynamics of the enhanced performing GLP-1/E2 conjugate. Furthermore, the superior benefits are not solely the consequence of a protracted time action despite subtle differences in exposure observed with the GLP-1/E2 conjugate relative to the GLP-1 analog. The body weight improvement observed with GLP-1/E2 treatment was completely abolished in global GLP-1R$^{-/-}$ mice and substantially blunted in CNS-specific GLP-1R$^{-/-}$ mice, which demonstrates the primary mechanism of action for body weight lowering resides in the CNS. The specific contribution of islet GLP-1 receptors to the activity of GLP-1/E2 is most likely involved in the glycemic benefits. Subsequent studies have shown the additive contribution of GLP-1/E2 on pancreatic islet function, cytoarchitecture, and protection from deleterious insults such as lipotoxicity (Schwenk et al., 2015; Tiano et al., 2015). Body weight lowering of GLP-1/E2 was partially ameliorated in estrogen receptor α and estrogen receptor β knockout mice. The contribution of membrane-anchored or membrane-embedded estrogen receptors remains to be determined. Further testing in mouse models with the knockout of estrogen receptors in select brain regions and islet cell populations would provide mechanistic insight into how these two hormones coordinately influence systemic metabolism.

Despite the powerful metabolic benefits associated with estrogen action, effects on the reproductive endocrine system and oncogenic potential have restricted the clinical use of estrogens to replacement therapy in

postmenopausal women. Importantly, GLP-1 receptors have not been reported to be expressed in these reproductive tissues, and, together with the complementary effects of GLP-1 and estrogen, support the logic that GLP-1–mediated targeting is an advantageous strategy. It appears to improve the therapeutic index of E2 and capture the benefit of more than one mode of action to positively affect metabolism. Biochemical signatures indicative of estrogen signaling were evident in those tissues and cells possessing GLP-1R expression, yet appreciably absent in cells without such receptor. Treatment with the stable GLP-1/E2 conjugate did not cause uterine hypertrophy in ovariectomized female rodents, whereas the labile conjugate, which increased circulating estrogen, caused significant uterine growth. Furthermore, the labile conjugate stimulated the proliferation of MCF-7 cells in vitro and accelerated growth of MCF-7 xenograft tumors in chronically treated mice as result of the systemically released estrogen. Unlike the labile conjugate, the stable GLP-1/E2 conjugate did not show tumorigenic toxicity, confirming the stability of the conjugate in circulation and that GLP-1 does not target the estrogen cargo to these cell types (Finan et al., 2012). More exhaustive toxicity studies are required to quantify the magnitude of improved therapeutic index for the stable GLP-1/E2 conjugate, including an examination of on-target and off-target effects in multiple species. Those cells that possess both GLP-1 and estrogen receptors are of noteworthy concern, as are pancreatic β-cells, as there is risk of promoting any pre-existing pancreatic tumors. As GLP-1 receptors are also broadly expressed, albeit at lower relative levels compared with what is observed in the CNS and pancreatic islets, there is potential to deliver unwanted estrogen at low levels and cause adverse effects. Independent from potential oncogenic or gynecologic toxicities, targeting neuronal circuitries involved in feeding behavior has risks as well. It is now evident that subsets of these neuronal populations involved in energy homeostasis are functionally connected to nonmetabolic, higher-order behaviors (Dietrich and Horvath, 2012). Despite evidence that GLP-1/E2 conveys positive effects on feeding behaviors such as reward and binge eating (Cao et al., 2014; Vogel et al., 2016), pharmaceutical agents with potent anorexigenic effects have shown adverse effects on behavior that include increased prevalence of depressive mood disorders (Christensen et al., 2007).

Although the medicinal benefits of the stable GLP-1/E2 conjugates have been demonstrated in these preclinical studies, many aspects of the molecular pharmacology and mechanism of action remain unresolved. In particular, the precise intracellular processing of the GLP-1/E2 conjugate that results in the release of an active estrogen cargo has not been determined, and, as such, the molecular identity that delivers estrogen activity remains unknown. Furthermore, whereas the estrogen appeared to have minimal impact on the pharmacokinetic profile of the GLP-1 conjugate and did not enhance the terminal half-life, it is still plausible to believe that the estrogen can alter the biodistribution of the conjugate to more privileged sites of CNS action, if only by enhancing brain penetration.

B. Glucagon-Mediated Delivery of Thyroid Hormone Tri-iodothyronine

Glucagon and thyroid hormone can individually promote weight loss and improve dyslipidemia in humans, which positions these two hormones as attractive candidates in development of a multifaceted medicine for treatment of cardiometabolic diseases. Many of the individual actions of these endogenous hormones overlap and suggest that a pairing could result in additional metabolic benefit. Thyroid hormones are classic mediators of multiple nodes of metabolic homeostasis due to diverse actions in broad tissues, as discussed early in this review. Primarily by hepatic action, thyroid hormone therapy can lower the circulating concentration of cholesterol and lipoproteins (Angelin and Rudling, 2010). In adipose depots, thyroid hormone can promote energy expenditure and lipolysis (Lin et al., 2015; Weiner et al., 2016). Central actions of thyroid hormone include hyperphagia and sympathetic outflow, which can also increase energy expenditure and cardiovascular adrenergic input (Lopez et al., 2010; Mittag et al., 2013). However, the adverse effects of excessive thyroid hormone are numerous and well-categorized, including cardiac hypertrophy, tachycardia, muscle catabolism, and bone deterioration. Despite the substantial metabolic attributes, thyroid hormone therapy must be dose titrated and carefully monitored, even in those receiving replacement therapy for thyroid deficiencies, including thyroidectomy.

Recognizing the benefits of liver-specific thyroid hormone action while attempting to mitigate the systemic toxicities, research has focused on chemical analogs with selective β receptor (TRb) selectivity, or analogs with preferential uptake in hepatocytes. The rationale of liver-selective thyromimetics was largely guided by studies showing enhanced hepatic presence and only trace expression in the heart. The chemical optimization to TRb selectivity was guided by molecular structures that identified different interactions of native thyroid hormones between the two predominant isoforms (Borngraeber et al., 2003; Bleicher et al., 2008). These medicinal chemistry refinements resulted in compounds that show favorable effects on lipoprotein profiles without influencing cardiac function, but body weight lowering was not observed and purportedly not expected based on hepatic action (Baxter and Webb, 2009). However, dose-dependent effects on hypothalamic-pituitary-thyroid axis suppression were evident for a few of these thyromimetics at higher doses. These effects were underscored in the reports (Erion et al., 2007), but now appear of appreciable importance. Possibly this biology is not structure or receptor specific, but instead identifies a previously

unrealized mechanism in hypothalamic-pituitary-thyroid feedback that originates in the liver. In this regard, unexpected toxicity pertaining to cartilage damage was observed in dogs following chronic treatment with a liver-selective thyromimetic eprotirome (Sjouke et al., 2014). It is unknown whether this is a class effect or something specific to this one TRb agonist. Consequently, there is a continued need for more thorough on-target toxicity studies with these thyromimetics. Possibly, these toxicology studies have been conducted, but yet to be reported, because they would have been required to support the advanced, unsuccessful clinical studies that terminated the development.

The peptide-based approach to delivery of estrogen seemed an attractive strategy for delivery of T3, and in particular its integrated use with glucagon. Glucagon receptors are highly concentrated in the liver, which is the preferred site for T3 action, but it is also present at low levels in adipose tissues, kidney, and throughout the cardiovascular system. These secondary sites represent areas for beneficial action to improve metabolism, but more importantly represent a risk for toxicity. A single molecule was designed with an equimolar equivalent of native T3 covalently conjugated to a DPP-IV–protected, C-terminally extended glucagon analog via a peptide spacer (Finan et al., 2016a) (Fig. 5). This design provided full inherent potency at the glucagon receptor for the T3 conjugate. Several control compounds were also generated to permit appropriate pharmacological comparisons. These additional peptides included the following: a conjugate with selective chemical substitution to the peptide to suppress glucagon activity, a compound with a linker that proved metabolically stable and was incapable of intracellular T3 release, and a third control conjugate that bore a metabolically-inert thyroid hormone.

The fully active glucagon/T3 conjugate restricted thyroid hormone action to tissues expressing the glucagon receptor with selective accumulation of T3 in the liver, which was confirmed in using labeled compound. Accumulation of thyroid hormone was not evident in tissues where its action was unwanted and devoid of glucagon receptor, most notably the heart and bone. Studies using glucagon receptor knockout mice confirmed the receptor selectivity in the conjugate and provides indirect evidence that the glucagon receptor is a necessary ingredient to T3 transport and biologic action. As a result of this hepatic-targeted biodistribution profile, the glucagon-T3 conjugate dose-dependently corrected dyslipidemia in various rodent models of dietary-induced metabolic syndrome, most notably mice fed HFD and Western-style diets (Finan et al., 2016a). Importantly, the benefits on lipid metabolism were muted when studied in mice with selective hepatic knockout of TRb, demonstrating the tissue and target selectivity of the conjugate. Evidence suggests that the weight-lowering efficacy of the conjugate can partially be governed by actions in adipose depots because glucagon receptors are present in rodent adipocytes, but to a much lesser degree than in liver. Indeed, the weight- and lipid-lowering effects of the glucagon/T3 conjugate can be partially attributed to uncoupling protein 1–mediated thermogenesis, enhanced FGF21 secretion, and biased PGC-1 cofactor signaling. Many agents have been shown to correct various forms of obesity in rodent models, but very few have shown reversal of arterial plaque deposition in rodent models of heart disease, and even fewer have reversed hepatic fibrosis in mouse models of nonalcoholic steatohepatitis. Emphasizing the translational aspects of combining the actions of these two hormones, intervention with this glucagon/T3 conjugate lessened arterial plaque area in diseased LDL receptor$^{-/-}$ mice, and also lessened fibrosis in mice with advanced fatty liver disease. These findings collectively demonstrate regenerative medicinal quality and enhanced safety of this specific hormone pair in cardiometabolic diseases.

In addition to enhancing the metabolic benefits of T3 by predominantly focusing its action at the liver via glucagon, this hormonal pair allowed for the reciprocal countersuppression of their individual inherent liabilities that restrict their individual medicinal use. The liver-directed thyroid hormone action offset the diabetogenic liability of glucagon, whereas the deleterious effects of thyroid hormone on cardiac muscle and its catabolic properties were minimal, indicating an improved therapeutic index. The magnitude of the improved therapeutic index will need to be more thoroughly studied to determine whether it is of sufficient magnitude to support chronic use in higher mammals, and most importantly humans. Although the original report provides compelling evidence about the potential benefits of glucagon-mediated targeting of thyroid hormone, progression toward clinical development will likely require chemical maturation. Further chemical refinement is possible and can be directed at fine-tuning the relative potency of the two hormones. Additionally, controlled metabolic stability of the conjugate, particularly the linker, may also be required to further enhance the potency and safety of the first reported conjugate candidate.

C. Glucagon-Like Peptide 1–Mediated Delivery of Dexamethasone

Chronic peripheral and central inflammation is a frequently reported feature of dietary-induced obesity and is commonly believed to play a causal role in the pathogenesis of the disease (Hotamisligil, 2006; Hotamisligil and Erbay, 2008; Gregor and Hotamisligil, 2011; Thaler et al., 2012, 2013). Although solid evidence supports a direct role of immunometabolic pathways in the development of obesity-linked insulin resistance (Hotamisligil et al., 1993, 1996; Hotamisligil and Spiegelman, 1994), therapeutic options to improve systems metabolism via counteracting obesity-associated inflammation

are scarce. Glucocorticoids are known for decades for their anti-inflammatory properties, but, as with other nuclear-acting hormones, their ubiquitous action profile limits their therapeutic utility and can lead to off-target effects. Expanding the concept of peptide-mediated nuclear hormone delivery, collaborative research of the DiMarchi/Tschöp laboratories recently let to the development of a molecule, which selectively restricts the action of dexamethasone to cells expressing the receptor for GLP-1, such as brain and the pancreas (Quarta et al., 2017) (Fig. 5). The GLP-1/dexamethasone chimera synergistically improved body weight in DIO mice, notably with superior metabolic action relative to treatment with GLP-1 or dexamethasone alone. In line with its action on key hypothalamic neurocircuits, weight loss induced by GLP-1/dexamethasone was a result of decreased food intake and increased energy expenditure and was associated with improved glucose metabolism and restored insulin sensitivity. Notably, the targeted delivery of dexamethasone to GLP-1R–positive cells prevented typical dexamethasone off-target effects on glucose handling, bone integrity, and hypothalamus-pituitary-adrenal axis activity (Quarta et al., 2017).

VII. Outlook

The integration of the small- and large-molecule pharmacology as exemplified in these first peptide–nuclear hormone conjugates is just a beginning of what could constitute a full class of novel drug candidates. The enhanced convenience in the infrequent administration offered by best-in-class GLP-1 agonists may not be ideal for drugs of this type. It is just one element that needs to be considered, as the preferential action profile of thyroid hormone or estrogen might require more intermittent dosing given the different pharmacodynamic manner in which nuclear hormones biochemically signal relative to a peptide acting at a surface receptor. Obviously, the primary consideration is performance to achieve the right balance between safety and efficacy, with convenience being an important but secondary consideration. These first reports of peptide-directed nuclear hormone pharmacology hold much promise, but it is best to maintain healthy scientific skepticism as the improvement in therapeutic index is quantified and relative risk–benefit in chronic treatment of multiple metabolic diseases is assessed.

So we come full circle with more than a century of experiences in search of medicinal agents that can provide the magnitude of metabolic improvement and weight lowering that has been demonstrated in the last decade with bariatric surgeries. It is a daunting challenge, exacerbated by the enormity of the public need and the growing realization of the personal and public consequences of chronic obesity. We can take confidence that we have never been better equipped scientifically to address the challenge, and medicinal advances in individually addressing cholesterol, glucose, and blood pressure are examples of what is possible. It seems inevitable that more than one solution will emerge and that each of them will require more than one mechanism of action. Possibly, what is most transformative in the emerging trend championed with peptide-based therapeutics is not the polyagonism, as combination therapy is a common feature in treating multiple chronic diseases. It is the use of hormones that were highly restricted in their use or, in fact, counterindicated. Glucagon may be the poster child where for more than half a century the focus has been exclusively on glucose and antagonism. When viewed in a more holistic sense, we now perceive how we can achieve indirect improvements in glucose through glucagon agonism associated with lower body weight when used in concert with a second or third hormone. We certainly do not want to repeat the errors of the rainbow pills in camouflaging toxicity. As such, the historical context that we present in this review and the body of literature it represents constitute a foundation for current and future research.

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Authorship Contributions

Wrote or contributed to the writing of the manuscript: Müller, Clemmensen, Finan, DiMarchi, Tschöp.

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