Outstanding Scientific Achievement Award Lecture 2011: Defeating Diabesity

The Case for Personalized Combinatorial Therapies

Matthias H. Tschöp[1] and Richard D. DiMarchi[2]

Matthias H. Tschöp, MD, from Munich, Germany, received the American Diabetes Association's prestigious 2011 Outstanding Scientific Achievement Award. The award was presented at the Association's 71st Scientific Sessions in San Diego, California in June 2011. The Outstanding Scientific Achievement Award recognizes distinguished scientific achievement in the field of diabetes, taking into consideration independence of thought and originality.

Currently holding the Alexander von Humboldt Professorship and Chair of Metabolic Diseases at the Technical University Munich as well as Director of the Institute of Diabetes and Obesity at the Helmholtz Center in Munich, Dr. Tschöp is internationally recognized for his work on how gut-brain communication regulates appetite and metabolism and for combining groundbreaking discovery with translational potential. A review of his peer-reviewed publication record and the breadth of his co-authors is a testament to his ability to build teams to advance science. Early in his career, Dr. Tschöp reported on the orexigenic, adipogenic, and metabolic effects of ghrelin. This added a pathway to the model of body weight and glucose control and established a novel set of drug targets for the treatment of the metabolic syndrome. His report of the efficacy of novel gut hormone coagonists targeting glucagon-like peptide 1 and glucagon receptors to reduce body weight and improve glucose tolerance broke new ground and offered novel clinical approaches for the treatment of diabetes and obesity. Dr. Tschöp is an influential voice explaining diabetes to the public and describing the scientific progress being made to improve lives.

Professor Richard D. DiMarchi is the Cox Professor for Chemistry and Gill Chair in Biomolecular Sciences at Indiana University and is Dr. Tschöp's key collaborator, providing transformative scientific vision and discovering a wealth of innovative molecules for their joint research successes toward novel therapeutics for the prevention and treatment of diabetes. Professor DiMarchi has invented several novel drugs that are in clinical use today, such as some of the first rDNA-based medicines, specifically Humalog and Forteo. He has been presented numerous awards including most recently the 2011 Bruce Merrifield Award of the American Peptide Society. The Tschöp and DiMarchi laboratories continue to work as a single integrated academic drug discovery unit as they have for the last 8 years, designing, validating, and optimizing new therapeutics for the treatment of diabetes and obesity.

THE CHALLENGE

Type 2 diabetes and obesity, often referred to as "diabesity," constitute two closely linked health threats of modern societies that continue to rise in prevalence despite decades of research investment (1). Numerous large and well-controlled studies have repeatedly shown that once patients establish significant obesity, no dietary or exercise regimen can restore and sustain a healthy body weight for any reasonably prolonged period in more than a tiny fraction of afflicted patients (2). Concurrently, intense worldwide efforts to discover and develop new drugs that might safely cure—or at least effectively minimize—diabesity have advanced at a frustratingly slow pace as the disease reached epidemic proportions. Regulatory agencies have not approved any new drug for the treatment of obesity since 1999 (the single exception being the European approval of rimonabant, a cannabinoid receptor antagonist/inverse agonist, which was subsequently terminated due to safety concerns) (3). In 1994, Jeffrey Friedman's discovery of leptin (4) destigmatized morbidly obese patients by proving that massively increased adiposity can be purely molecular and not a function of education or willpower. Despite leptin treatment failing to cure human obesity, its discovery triggered an explosion of molecular research to find another "silver bullet" to cure diabesity. Multiple other intriguing peptides and proteins emerged as drug candidates, including glucagon-like peptide 1, amylin, adiponectin, ghrelin, resistin, glucose-dependent insulin stimulatory peptide, peptide YY(3-36) (PYY(3-36)), and oxyntomodulin (5). Yet in spite of numerous promising early results with these hormones and a progressively more sophisticated understanding in the biochemical basis of metabolic control, the silver bullet has yet to emerge.

Possibly the urgent need for therapy is demanding too much, too soon, of researchers in drug discovery and development today. As an example, it took more than a decade to appreciate the recently discovered role for leptin in control of glucose independent of body weight change, along with its potential for use in the treatment of type 1 diabetes (6). The prospect for a breakthrough, possibly a diabesity cure, has most recently been reinvigorated by the discovery of irisin, a protein secreted by skeletal muscle that controls thermogenesis in brown adipose tissue (7).

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DOI: 10.2337/db12-0272
Figure 3 animation is available online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db12-0272/-/DC1.
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We have become increasing appreciative that within the "one molecule" approach, some of the above mentioned pathways might deliver sizable efficacy in specific patient subpopulations, but they demonstrate mediocre efficacy and considerable safety concerns when studied in broad, undefined patients suffering from obesity and diabetes. New technology to enhance the traditional characterization of sex, age, and race to better segregate patients is as much needed as the new therapeutics. Identification of such subpopulations is likely to promote the re-examination of previously promising but fallen therapeutics tailored for study in more appropriate patient subgroups. This progression toward a more personalized approach in metabolic medicine is ongoing and offers hope that the eventual treatment of diabesity might constitute a collection of silver bullets tailored for specific populations.

The pursuit of effective and safe medicines for the treatment of diabetes and obesity has been further complicated by a rapidly changing drug development landscape and a harsh regulatory environment. The total number of large pharmaceutical companies with the resources to finance and conduct large, long-term, multicenter, phase 3 clinical trials required for drug approval has been decimated. This select community that numbered more than 40 in the early 1990s is barely a dozen today with a continuing downward trend (Fig. 1). Consolidation does not stimulate innovation; quite to the contrary, major mergers and acquisitions foster uncertainty that slow clinical development. The remaining pharmaceutical companies are struggling to keep their drug candidate portfolios filled, while simultaneously reducing their workforce to deal with economic realities. The steady transition of large pharmaceutical companies from research and development to acquisition and development operations accelerates the importance of forging a healthy balance of internal investments with academic research institutions and flexible biotech companies. In addition, it can be expected that global regulatory agencies will act with particular caution when judging the approval of novel obesity drugs. Any significant adverse effect or health risk associated with a novel obesity medicine will obviously be difficult to endorse if only minor metabolic benefits without constructive impact on survival are observed. The irony is that some of the candidate therapeutics may offer benefits, which, apart from improved BMI and lowered HbA_{1c}, might appreciably reduce cancer risk, cardiovascular disease, or neurodegenerative diseases. However, such discoveries will not occur in an environment that does not appropriately balance risk with reward. Bringing forth such drugs is a huge undertaking that is steadily growing to a point where even the largest of the large companies may be unable to tolerate the risk, leaving the task for governments. This would constitute a most unfortunate development for governments since the economic burdens of obesity and the associated diseases represent a huge economic burden. Without the skills inherent to pharmaceutical companies and the creativity that most typically resides external to government institutions, the path to a cure will be less likely and of greater length. It is the work of this generation of scientists, clinicians, financiers, and politicians to define a win-win environment where creativity can flourish. This is the health care challenge of our age, and the consequence of insufficient progress may prove as dangerous as that of moving with excessive speed.

FIG. 1. After two decades of decimation, only a few large pharmaceutical companies remain capable of conducting multiple parallel phase-3 drug trials (boldface indicates companies that are currently still in operation). The downward trend continues and may turn into a bottleneck for late-stage development of potential diabetes and obesity breakthrough drugs.

THE OPPORTUNITY

The only currently available interventions with curative potential to treat diabesity are bariatric surgeries (8). Such surgical procedures for the treatment of morbid obesity include Roux-en-Y gastric bypass (RYGB), vertical sleeve gastrectomy (VSG), and others. The procedures are rising in popularity because both RYGB and VSG lead to massive weight loss, even weight normalization, in most cases. But there are some cases where nonresponsiveness and weight regain have been reported. Obviously, these procedures are highly invasive, irreversible, and associated with considerable risk, especially considering the high BMI and the combination of comorbidities a typical surgical patient suffers from. The very nature of the procedure speaks volumes about the need for effective therapy and the health and lifestyle consequences of this disease. Nonetheless, these surgeries instill hope and potential for metabolism researchers around the world. This hope stems from the observation that at least some bariatric surgeries, such as RYGB or VSG, lead to rapid metabolic improvements. In some cases, diabetes is cured long before any significant amounts of weight loss occur (9). No clear molecular mechanism has been confirmed so far, but there is hardly any metabolic disease institute or research center that does not engage in some research activity to discover the underlying pathway (s) driving these metabolic improvements. Once these mechanisms are identified, achieving comparably impressive body weight loss and glucose improvement not with surgery, but instead with pharmacology, becomes ever more feasible (10).

Parallel development that was propelled by the realization of leptin's actions in the brain (11) deepen the appreciation that the central nervous system (CNS) not only controls appetite, but also plays a major comodulatory role in the management of systemic metabolism. A complex network of specific hypothalamic, midbrain, and hindbrain circuits constantly receives afferent information pertaining to nutrient availability and metabolic status from gut, liver, adipose, and other metabolically relevant tissues. These hormonal, nutrient, and neuronal signals are processed centrally with sensory input from the environment, such as olfaction, taste, or visual information. A measured central

response in the form of efferent molecular signals is returned to metabolically relevant tissues and organs to orchestrate systemic homeostasis via coregulation of behavioral and metabolic processes (12). It appears that there is some level of redundancy in CNS processing of afferent information as it typically responds most efficiently to distinct patterns rather than to changes in a single peripheral signal, for example, altered circulating concentrations of the adiposity promoting stomach hormone ghrelin (13).

One of the more popular hypotheses regarding the "mysterious" molecular basis of surgically induced improvements in adiposity and metabolism is that the aforementioned afferent signaling patterns to the CNS are modified by the surgical procedure. Support for such belief comes from the observation that blood concentrations of gut hormones, which regulate systemic metabolism and act at least in part in the CNS (such as glucagon-peptide 1 [GLP-1], ghrelin, amylin, or PYY(3–36), rapidly improve from impaired levels typically associated with obesity and type 2 diabetes (14). Importantly, all of these afferent hormones target receptor centers, which are found in the midst of brain circuits that potently control appetite and spontaneous locomotor activity as well as systemic metabolism. There may be another inherent advantage to focusing on afferent metabolic hormones that signal through receptors in key CNS control centers: The alternative approach to directly targeting CNS neuronal communication has not proven successful with the largest limitation being the difficulty in achieving specificity sufficient to sustain efficacy without adverse pharmacology. Neurotransmitters and neuropeptides have demonstrated impressive efficacy through action at key regulatory centers such as the hypothalamus, but promiscuous activity in other brain areas eventually promoted therapeutic complications. By harnessing nature's molecular tools and utilizing a combination of afferent hormones exclusively targeting select metabolic control systems in the CNS, the development of novel therapeutics of unprecedented efficacy and safety may be possible. This vision aligns with the phenotypic molecular profiling observed with bariatric surgery where metabolic benefits are increasingly believed to result from changes in CNS hormonal signaling that constructively influence body weight and metabolism.

It follows that some combination of afferent signals could offer great potential for the treatment of obesity and type 2 diabetes. Scientists at Amylin Pharmaceuticals have reported seminal observations supporting this belief. They demonstrated in rodents and humans that combined treatment with parallel injections of the pancreatic hormone amylin and leptin synergistically enhanced body weight loss and metabolism to an extent not possible with monotherapy (15). The purported mechanism of action favored a specific and unique biochemical interaction among the amylin and leptin receptor signaling pathways. While that interaction remains an intriguing possibility, recent results from studies in our laboratories reveal that combinations of GLP-1 with leptin or fibroblast growth factor 21 seem to deliver comparable synergistic effects in obese rodent models (16). Intriguingly, we find that reversing leptin resistance with these hormone combinations cannot be achieved by comparable matched weight loss through caloric restriction (16). The molecular basis for this unique pharmacological virtue and whether it is identical when using different hormone combinations remains an objective of current studies. The translational benefit to humans could be enormous, especially when viewed in the light of the first translational success with amylin and leptin.

These initial results using combinations of two separate hormones hold huge promise, but the complexity of human study with multiple experimental agents cannot be understated. Identification of the preferred hormone combinations to constructively stimulate afferent signaling in specific patient subpopulations will likely require iterative, empirical combinations, potentially requiring more than two parallel signals. This path while scientifically rationale will render commercial development as a route to eventual approval as drug treatment for diabesity nearly impossible. Consequently, combination of several signals into a single molecule can streamline the development task, but more importantly it can offer unique pharmacological virtues. With this as foundational thinking, we recognized that changes in food intake are coordinately compensated by changes in energy expenditure. We therefore hypothesized that in order to achieve maximum body weight loss at least one thermogenesis-inducing component should ideally be paired with at least one satiety-inducing component in a single molecule. We favor macromolecules such as endogenous peptide and protein hormones since they are of inherently high potency and provide sufficient molecular size to engineer agonism at multiple receptors without excessive change to native conformation. This latter feature minimizes the risk for adverse effects and, in particular, immunogenicity.

The incretin hormone GLP1, which also targets CNS neurons, was chosen as one component since it has the proven capability to lower glucose and increase satiety (17). Although completely counterintuitive at the time of our selection, we chose glucagon as the other component for our first single molecule coagonist. Unopposed, chronic glucagon agonism triggers lipolysis and promotes thermogenesis (18). We explored whether the diabetogenic liability inherent to excessive glucagon agonism could be buffered by simultaneous GLP-1 agonism while achieving the combinatorial virtue of each hormone in accelerating body weight reduction. Numerous coagonists with differing ratios of glucagon versus GLP-1 agonism were designed and synthesized followed by extensive testing by in vitro and in vivo methods (19) (Fig. 2). The results were overwhelmingly positive as these glucagon-GLP-1 coagonists achieved unprecedented weight loss in obese rodents at low doses, and without any apparent adverse effects. Glucose tolerance and insulin sensitivity normalized at an accelerated pace when compared with therapy with monoagonists. The metabolic benefits resulting from weight loss overwhelmed any diabetogenic liability inherent to chronic, unopposed glucagon agonism. Only a modest relative amount of GLP-1 agonist was needed to buffer against the danger of excessive glucagon action (19). These results were independently confirmed by studies at Merck Research Laboratories working with lower potency coagonists based upon oxyntomodulin (20). GLP-1–glucagon coagonist drug candidates are advancing in clinical study and, possibly more than anything, they are demonstrating how proverbial "out of the box" thinking has liberated us to utilize glucagon in a manner completely opposite to what textbook wisdom taught us about its action in promoting diabetes.

These observations encouraged the design and synthesis of a second counterintuitive coagonist. In this instance the second hormone of interest relative to GLP-1 was

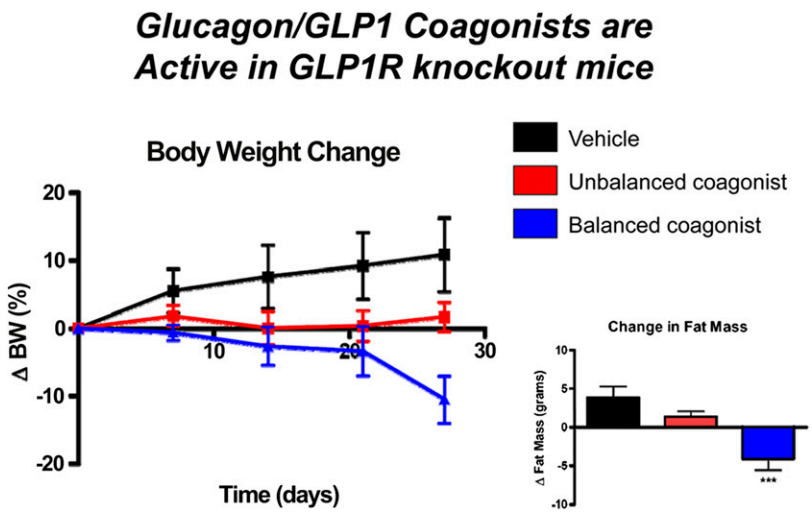

FIG. 2. Proof of metabolic in vivo activity beyond GLP-1 agonism in coagonists with additional imbalanced and balanced glucagon coagonism. Effects on body weight and body fat in diet-induced obese mice genetically engineered to lack the receptor for GLP-1. BW, body weight. ***$P < 0.001$. Modified from ref. (19).

gastrointestinal polypeptide glucose-dependent insulinotropic polypeptide (GIP). Common wisdom had directed attention to the development of GIP antagonists for the treatment of diabesity based upon study with a putative GIP antagonist (21) and separate work using targeted mouse mutagenesis where the GIP receptor gene had been disrupted (22). Despite the rodent reports, it seemed inconsistent to us that a hormone and its receptor that were homologous to GLP-1 would not be similarly constructive in glucose and possibly body weight control, given their purported roles in physiology. We prepared and studied high potency, well-balanced, long-acting GLP-GIP coagonists. They showed similarly impressive efficacy and metabolic benefits in obese rodents (data not provided) to that which we had previously observed with GLP-1–glucagon coagonists. Tangentially, it should be noted that in the course of the work we observed the purported GIP antagonist used to originally validate the need for GIP antagonism to be a weak GIP agonist. In addition, the translation of the observations with GIP receptor knockout mice was questioned by similar prior results that had been observed with GLP-1 receptor knockout mice. We conclude that there is no substitute for pharmacology with well-characterized molecular probes when making predictions about drug development for the treatment of human diseases.

We have since continued our search for novel combinatorial therapeutics. Most recently we have extended our observations with independent GLP-1 coagonists through the discovery of high potency, balanced triagonists. These triagonists are single peptides of molecular size comparable to the mono- and coagonists. They possess a single binding face that is promiscuous for the GLP-1, glucagon, and GIP receptors. These GLP-1-glucagon-GIP triagonists have proven superior to each of the coagonists in the treatment of obese mice and rats (data not provided). They have the independent virtues of each hormone, and importantly the liabilities are muted by each other's virtue. In a comparative sense there is an apparent ability to achieve superior efficacy relative to monoagonism, lessening the temptation to overly agonize a single pathway. We like to believe—although we have certainly not yet proven—that we have discovered the wisdom of nature in using polypharmacy to achieve chronic, safe, efficacious therapy through partial, or full, concerted agonism at multiple receptors. From a medicinal perspective, it is important to acknowledge that the chemical composition of these novel agents is unimolecular, and each peptide cannot lead to simultaneous occupation of two different receptors or heterodimerization in the manner common to dimers or high molecular forms. Rather a single peptide has been carefully engineered to serve as a "master key" for several receptors, while retaining the size and to a large extent the native sequence of the natural hormones (Fig. 3).

RISKS AND LIMITATIONS

In spite of some enlightened arguments and encouraging preclinical data, it certainly would be naïve not to acknowledge the difficult path forward in treating human obesity and diabetes. While natural peptides have frequently proven themselves to be miraculous medicines with minimal off-target toxicity, the potential for dose-limiting, on-target toxic side effects remains a possibility. Insulin serves as an excellent example of the unprecedented efficacy but life-threatening risk with excessive agonism. Another potential danger is the inherent crossreactivity of single molecule polyagonists increasing the potential for unintended effects through individual receptors or some synergy in undesired signaling. A final obvious reservation is the acceptance that studies in mice and rats do not always predict efficacy or toxicity in humans. Will chronic glucagon agonism function equally in humans as it has in rodents, and furthermore is the preferred ratio for minimizing adverse effects common across species? One point to note in that regard is the unusually progressive wasting syndrome observed in patients suffering with glucagonomas (23).

Yet even an advanced approach such as single molecule co- and triagonists will benefit from—and quite possibly depend upon—advances in deep phenotyping of patients with diabesity. We will need to define and identify subgroups that would benefit most from distinct personalized drugs, as well as the prospect that different agents might

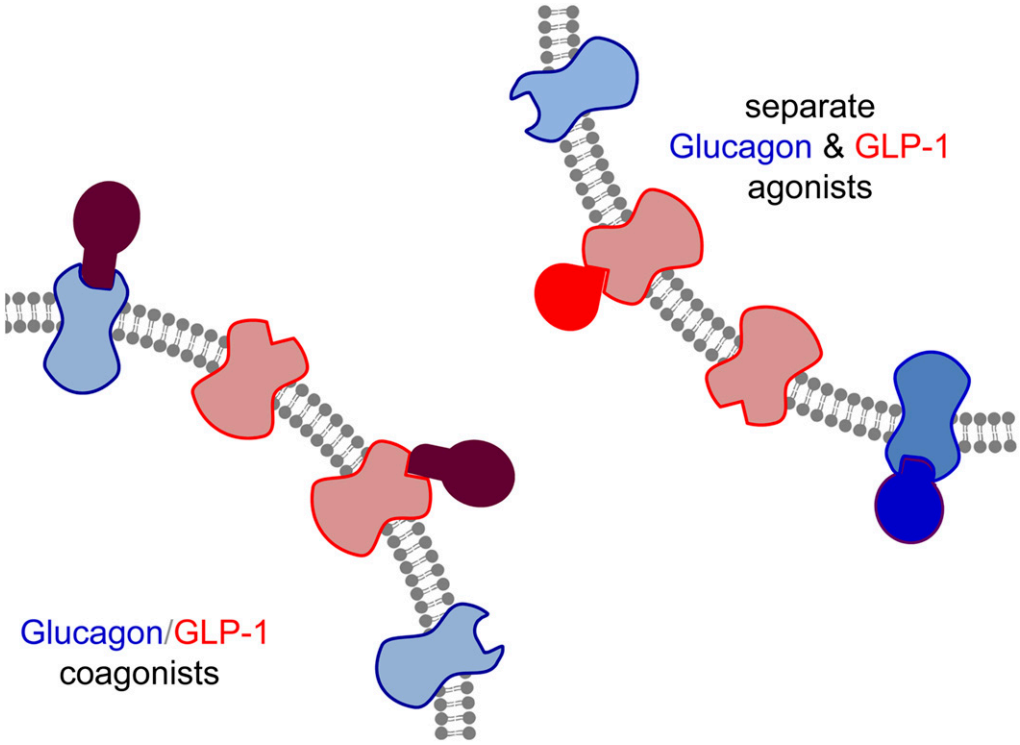

FIG. 3. Principle of novel drug candidates for the treatment of diabetes and obesity based on single molecule peptide coagonism. A single natural peptide hormone (in this example, glucagon) is engineered using minimal chemical changes to turn it into a "master key," which activates additional receptors (as in this example, GLP-1R). The size of the resulting molecule is similar to that of a single agonist peptide (e.g., GIP), rather than a two-peptide chimera. Additional changes can convey protection from protease cleavage and add longer lasting activity by changing pharmacokinetic profiles. (Fig. 3 animation is available online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db12-0272/-/DC1.)

serve the same patient differently at different points in the treatment of their disease (24). For example, reliable predictive characterization on GLP-1 responders and nonresponders within patient populations with diabetes and obesity could help to predict suitable therapy and minimize the risk of an inappropriate therapeutic prescription. Undoubtedly, efficient parallel progress toward multiple choices of clinically tested single molecule polyagonists along with a deeper patient phenotyping will enable a more personalized metabolic prescription and represent our best chance for a nonsurgical management of diabesity.

We are in our infancy in formulating the best medicinal approach to treat the global epidemic of disease. The integration of drug therapy to facilitate less invasive surgical procedures (such as gastric banding) may offer synergistic potential. Furthermore, single molecule drug combinations are likely not to be limited to macromolecule combinations. Peptide and protein-based targeted delivery of more traditional small molecule medicines holds great potential for enhanced efficacy. In particular, we favor single molecule combinations of peptides with nuclear hormones as the effect of the latter can be highly targeted to select tissues that possess the peptide receptor as a prerequisite to accessing the nuclear-location hormone receptor. We have most recently explored such single molecule combinations as GLP-1 and steroids to achieve sizable expansion in efficacy without the hallmark toxicities frequently mediated by nuclear receptors (data not provided).

Healthy skepticism is an important element in drug discovery, especially when one is aiming to cure a disease for which decades of research have failed to achieve the needed breakthrough. Frequently, the argument is made that redundancy among the considerable number of circulating signals regulating metabolism render it impossible to design a drug that can continuously lower body fat and improve glucose tolerance. The argument is grounded in the so-called thrifty gene hypothesis, which professes that our genetic constitution was shaped to survive long periods under variable and uncertain caloric supply (25). Therefore, multisignal-based efficiency might have developed in order to optimally store calories and defend body fat, although the role of the thrifty gene hypothesis for diabetes has recently been challenged (26). If securing calorie intake and maintaining caloric storage are so important for the survival of our species than it seems plausible that pharmacological intervention may be wishful thinking. This could be the case, but we should recognize that endocrinologists have been down this path before. Contraceptives were developed by pharmacologically modifying one or several afferent signals in "tricking the brain to perceive pregnancy" when none existed, thereby shutting down parts of the reproductive system (27). Since reproduction is seminal to survival of the species, there is hope in analogy that we might just be equally fortunate in replicating one more time a similar strategy to defeat diabesity.

ACKNOWLEDGMENTS

R.D.D. is a consultant for and receives research funds from Hoffmann-La Roche and Merck and is co-founder, SAB Member, and shareholder at Ambrx, Inc. M.H.T. is a consultant for and receives research funds from Hoffmann-La Roche. No other potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Olshansky SJ, Passaro DJ, Hershow RC, et al. A potential decline in life expectancy in the United States in the 21st century. N Engl J Med 2005;352:1138–1145
2. Makris A, Foster GD. Dietary approaches to the treatment of obesity. Psychiatr Clin North Am 2011;34:813–827
3. Aronne LJ, Powell AG, Apovian CM. Emerging pharmacotherapy for obesity. Expert Opin Emerg Drugs 2011;16:587–596
4. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–432
5. Greenwood HC, Bloom SR, Murphy KG. Peptides and their potential role in the treatment of diabetes and obesity. Rev Diabet Stud 2011;8:355–368
6. Hedbacker K, Birsoy K, Wysocki RW, et al. Antidiabetic effects of IGFBP2, a leptin-regulated gene. Cell Metab 2010;11:11–22
7. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012;481:463–468
8. DeMaria EJ. Bariatric surgery for morbid obesity. N Engl J Med 2007;356:2176–2183
9. Bayham BE, Greenway FL, Bellanger DE, O'Neil CE. Early resolution of type 2 diabetes seen after Roux-en-Y gastric bypass and vertical sleeve gastrectomy. Diabetes Technol Ther 2012;14:30–34
10. Tam CS, Berthoud HR, Bueter M, et al. Could the mechanisms of bariatric surgery hold the key for novel therapies? Report from a Pennington Scientific Symposium. Obes Rev 2011;12:984–994
11. Stephens TW, Basinski M, Bristow PK, et al. The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature 1995;377:530–532
12. Nogueiras R, Tschöp MH, Zigman JM. Central nervous system regulation of energy metabolism: ghrelin versus leptin. Ann N Y Acad Sci 2008;1126:14–19
13. Tschöp MH, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. Diabetes 2001;50:707–709
14. Laferrère B, Reilly D, Arias S, et al. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. Sci Transl Med 2011;3:80re2
15. Roth JD, Roland BL, Cole RL, et al. Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies. Proc Natl Acad Sci USA 2008;105:7257–7262
16. Müller TD, Sullivanc LM, Habegger K, et al. Restoration of leptin responsiveness in diet-induced obese mice by an optimized leptin analog in combination with exendin4 or FGF21. J Pept Sci. In press
17. Barrera JG, Sandoval DA, D'Alessio DA, Seeley RJ. GLP-1 and energy balance: an integrated model of short-term and long-term control. Nat Rev Endocrinol 2011;7:507–516
18. Holloway SA, Stevenson JA. Effect of glucagon on oxygen consumption in the cold-adapted rat. Can J Physiol Pharmacol 1964;42:860–862
19. Day JW, Ottaway N, Patterson JT, et al. A new glucagon and GLP-1 coagonist eliminates obesity in rodents. Nat Chem Biol 2009;5:749–757
20. Pocai A, Carrington PE, Adams JR, et al. Glucagon-like peptide 1/glucagon receptor dual agonism reverses obesity in mice. Diabetes 2009;58:2258–2266
21. McClean PL, Irwin N, Cassidy RS, Holst JJ, Gault VA, Flatt PR. GIP receptor antagonism reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat diet. Am J Physiol Endocrinol Metab 2007;293:E1746–E1755
22. Hansotia T, Maida A, Flock G, et al. Extrapancreatic incretin receptors modulate glucose homeostasis, body weight, and energy expenditure. J Clin Invest 2007;117:143–152
23. Klein S, Jahoor F, Baba H, Townsend CM Jr, Shepherd M, Wolfe RR. In vivo assessment of the metabolic alterations in glucagonoma syndrome. Metabolism 1992;41:1171–1175
24. Hamburg MA, Collins FS. The path to personalized medicine. N Engl J Med 2010;363:301–304
25. Neel JV, Weder AB, Julius S. Type II diabetes, essential hypertension, and obesity as "syndromes of impaired genetic homeostasis": the "thrifty genotype" hypothesis enters the 21st century. Perspect Biol Med 1998;42:44–74
26. Speakman JR. Thrifty genes for obesity, an attractive but flawed idea, and an alternative perspective: the 'drifty gene' hypothesis. Int J Obes (Lond) 2008;32:1611–1617
27. Lunenfeld B, Sulimovici S, Rabau E. Mechanism of action of anti-ovulatory compounds. J Clin Endocrinol Metab 1963;23:391–394