Christoffer Clemmensen,[1] Joseph Chabenne,[2] Brian Finan,[1] Lorraine Sullivan,[2] Katrin Fischer,[1] Daniela Küchler,[1] Laura Sehrer,[1] Teja Ograjsek,[3] Susanna M. Hofmann,[3] Sonja C. Schriever,[1] Paul T. Pfluger,[1] Jason Pinkstaff,[2] Matthias H. Tschöp,[1] Richard DiMarchi,[4] and Timo D. Müller[1]

GLP-1/Glucagon Coagonism Restores Leptin Responsiveness in Obese Mice Chronically Maintained on an Obesogenic Diet

We recently reported restoration of leptin responsiveness in diet-induced obese (DIO) mice using a pharmacologically optimized, polyethylene-glycolated (PEG)-leptin analog in combination with exendin-4 or FGF21. However, the return of leptin action required discontinuation of high-fat diet (HFD) exposure. Here we assess whether a single peptide possessing balanced coagonism at the glucagon-like peptide 1 (GLP-1) and glucagon receptors can restore leptin responsiveness in DIO mice maintained on a HFD. DIO mice were treated with PEG-GLP-1/glucagon (30 nmol/kg every fourth day) to induce an ~15% body weight loss, upon which they were randomized to continue PEG-GLP-1/glucagon therapy or reassigned to receive supplemental daily PEG-leptin (185 nmol/kg/day). The addition of PEG-leptin to PEG-GLP-1/glucagon resulted in an ~18% greater weight loss as compared with PEG-GLP-1/glucagon alone and was accompanied by further decreases in food intake and improved glucose and lipid metabolism. The beneficial effect of PEG-leptin supplementation occurred after an initial body weight loss similar to what we previously reported following reduced dietary fat along with PEG-leptin and exendin-4 or FGF21 cotreatment. In summary, we report that GLP-1/glucagon coagonism restores leptin responsiveness in mice maintained on a HFD, thus emphasizing the translational value of this polypharmacotherapy for the treatment of obesity and diabetes.

Diabetes 2014;63:1422–1427 | DOI: 10.2337/db13-1609

Pharmacological approaches to combat obesity are hampered by limited efficacy or considerable adverse effects. Therefore the development of safe and efficient pharmacotherapies is an increasing global priority. The adipocyte hormone leptin plays a pivotal role in energy metabolism due to its ability to decrease body weight by inhibiting food intake and increasing energy expenditure. Since its discovery (1), leptin has been extensively studied for its potential in weight management. However, where leptin replacement promotes weight loss in congenitally leptin-deficient obese rodents (2,3) and humans (4–6), its potential as a stand-alone therapy under conditions of diet-induced obesity is limited due to leptin resistance (7,8). However, leptin responsiveness can be

[1]Institute for Diabetes and Obesity, Helmholtz Center Munich, German Research Center for Environmental Health, Technical University Munich, Munich, Germany
[2]Ambrx, Inc., San Diego, CA
[3]Institute for Diabetes and Regeneration, Helmholtz Center Munich, German Research Center for Environmental Health, Ludwig-Maximilian University Munich, Munich, Germany
[4]Department of Chemistry, Indiana University, Bloomington, IN

Corresponding authors: Matthias H. Tschöp, matthias.tschoep@helmholtz-muenchen.de, and Richard DiMarchi, rdimarch@indiana.edu.

Received 18 October 2013 and accepted 18 December 2013.

This article contains Supplementary Data online at http://diabetes.diabetesjournals.org/lookup/suppl/doi:10.2337/db13-1609/-/DC1.

restored using pharmacology (9–11) and suggests that leptin administration may hold promise as a valuable adjunct in novel combinational pharmacotherapies (12,13). Accordingly, coadministration of leptin with the pancreatic peptide amylin restores leptin responsiveness and synergistically decreases body weight in diet-induced obese (DIO)-prone rats and in calorie-restricted obese humans (9). Similar results were observed in DIO mice by coadministration of polyethylene-glycolated (PEG)-leptin with exendin-4 or FGF21 (10). In both studies, adjunctive administration of the respective peptides with leptin synergistically improved weight loss relative to treatment with the peptides alone. However, both studies required a moderately low content of dietary lipids to observe a return of leptin responsiveness. These data align with a growing number of reports indicating that dietary macronutrients, especially fat and sugar, are detrimental to leptin sensitivity even before the onset of obesity or hyperleptinemia (14–19). Restoration of leptin responsiveness in the context of a chronic obesogenic environment has yet to be demonstrated. This challenge has led us to explore the potential of glucagon-like peptide 1 (GLP-1)/glucagon coagonism, which normalizes adiposity without the necessity of dietary change (20), as a complement to leptin-based therapy.

Our results show that PEG-GLP-1/glucagon restores leptin responsiveness and, when combined with PEG-leptin, synergistically improves body weight loss in DIO mice continuously exposed to a high-fat diet (HFD). The regain of leptin action occurred after an initial weigh loss that is similar to previous reports (10) following reduced dietary fat. The return of leptin responsiveness was reflected by enhanced weight loss induced by adjunctive PEG-leptin treatment, which was mediated by decreased food intake and accompanied by improved glucose and lipid metabolism.

RESEARCH DESIGN AND METHODS

PEG-Leptin and PEG-GLP-1/Glucagon Coagonist

The synthesis, purification, and characterization of PEG-leptin and PEG-GLP-1/glucagon were described previously (10,20), and both compounds were used without any further chemical modification or change in formulation.

Animals and Diet

Six- to eight-week-old male C57BL/6 mice were maintained at 23 ± 1°C, constant humidity, and a 12-h light–dark cycle. Mice had free access to water and were fed ad libitum with a HFD comprising 58% of calories from fat (D12331; Research Diets, New Brunswick, NJ). All procedures were approved by the Animal Use and Care Committee of Bavaria, Germany.

In Vivo Evaluation of PEG-GLP-1/Glucagon and PEG-Leptin Cotreatment

Mice were treated via subcutaneous injection (5 μl/g body weight) as indicated. Body composition was analyzed using a magnetic resonance whole-body composition analyzer (EchoMRI, Houston, TX). For glucose tolerance, levels of blood glucose were sampled from 6-h fasted mice following intraperitoneal administration of 1.5 g glucose per kilogram body weight.

Biochemical Analysis

For tissue collection, mice were fasted for 4 h and treated with the compounds 2 h prior to sample collection. Plasma levels of insulin (Crystal Chem, Inc., Downers Grove, IL), cholesterol (Wako Chemicals, Neuss, Germany), leptin (ALPCO Diagnostics, Salem, NH), and adiponectin (Millipore, Schwalbach, Germany) were measured using commercially available kits according to the manufacturers' instructions. Levels of liver triglycerides were assessed as previously described (21).

Gene Expression Analysis

Gene expression was profiled with quantitative real-time RT-PCR–based techniques using TaqMan single probes (n = 6–7 mice per group) or TaqMan low-density arrays (n = 4–6 mice per group) (Applied Biosystems, Germany). The relative expression of the selected genes was normalized to the housekeeping gene *Hprt* or *Rpl27*.

Statistical Analysis

Differences between treatment groups were assessed by one- or two-way ANOVA followed by post hoc comparison or Student two-tailed unpaired t test. All results are given as mean ± SEM. $P < 0.05$ was considered statistically significant.

RESULTS

GLP-1/Glucagon Coagonism Restores Leptin Responsiveness During Chronic HFD Exposure

Male 24-week-old DIO mice (52.69 ± 0.68 g) were treated for 9 days with the PEG-GLP-1/glucagon coagonist (30 nmol/kg every fourth day) or vehicle control. Similar to previous reports (20), treatment with PEG-GLP-1/glucagon resulted in decreased body weight relative to mice treated with vehicle control (−14.46 ± 1.26 vs. −2.07 ± 0.72%; $P < 0.001$) (Fig. 1*A*). The decreased body weight was the result of a decrease in body fat and lean tissue mass and was accompanied by lower food intake and improved glucose tolerance relative to mice treated with vehicle control (all $P < 0.001$) (Fig. 1*B–E*).

At study day 9, mice treated with the coagonist were continued on either PEG-GLP-1/glucagon or assigned to receive additional daily PEG-leptin (Fig. 2). As negative controls, mice that were initially treated with vehicle were rerandomized to receive either daily vehicle or PEG-leptin (185 nmol/kg/day) alone. Daily adjunctive administration of PEG-leptin and PEG-GLP-1/glucagon led to a significantly greater reduction in body weight as compared with mice treated with PEG-GLP-1/glucagon alone (Δ body weight at study day 33 relative to day 0 was −44.05 ± 2.83 (−23.68 ± 2.17) vs. −26.49 ± 4.93% (−13.93 ± 2.67 g); $P < 0.05$) (Fig. 2*A*). The enhanced weight loss in mice treated

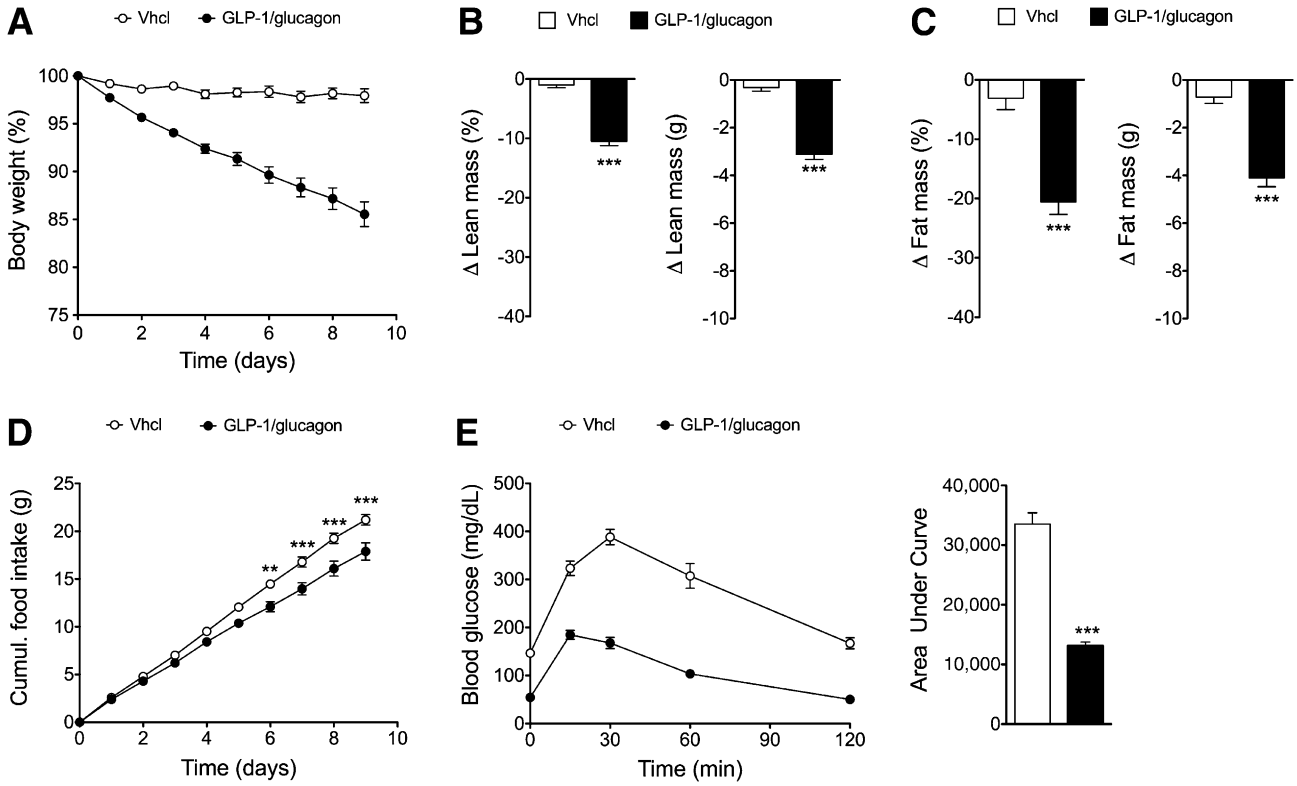

Figure 1—Effect of PEG-GLP-1/glucagon monotherapy on energy and glucose metabolism in DIO mice. (*A*) Percentage body weight, (*B* and *C*) change of body composition, (*D*) cumulative food intake, and (*E*) glucose tolerance of DIO mice treated chronically every 4 days with either vehicle (PBS; $n = 14$) or PEG-GLP-1/glucagon (30 nmol/kg/4 days; $n = 21$). Injections were given subcutaneously at study days 0, 4, and 8. Glucose tolerance (intraperitoneal; 1.5 g glucose/kg body weight) was measured at study day 8. Changes in body composition reflect changes from study day 0 to 8. Data represent means ± SEM. $^{**}P < 0.01$; $^{***}P < 0.001$; Vhcl, vehicle; Cumul., cumulative.

with PEG-GLP-1/glucagon and PEG-leptin was associated with decreased body fat mass, reduced food intake, and improved glucose tolerance and insulin sensitivity relative to mice maintained on PEG-GLP-1/glucagon alone (all $P < 0.05$) (Fig. 2*B–F*). Together, these data indicate that GLP-1/glucagon coagonism restores leptin responsiveness under persistent HFD exposure. Of note, the beneficial effect of PEG-leptin occurred after an initial body weight loss of ~15% relative to day 0 (Fig. 2*A*). The return of leptin responsiveness at this degree of weight loss is similar to what we previously reported in DIO mice treated with the combination of PEG-leptin and exendin-4 or FGF21 and following switch to conventional chow diet (10).

Consistent with the observation that leptin decreases body weight and food intake when added to the PEG-GLP-1/glucagon coagonist, we also found improved measures of glucose and lipid metabolism in these mice. Mice treated with PEG-GLP-1/glucagon alone, as compared with vehicle-treated controls, showed lower levels of liver triglycerides and decreased plasma levels, cholesterol, and endogenous leptin, whereas plasma levels of insulin and adiponectin were unchanged (Supplementary Fig. 1*A–E*). Except for adiponectin, all of these biochemical measures, including insulin, were improved by the addition of PEG-leptin (Supplementary Fig. 1*A–E*). The reduced cholesterol levels were also accompanied by a trend for decreased apolipoprotein B48 (Supplementary Fig. 1*F*), a profound decrease in leptin mRNA levels in the epididymal white adipose tissue, and a decrease in hypothalamic expression of the leptin receptor (Supplementary Fig. 1*G* and *H*). No differences in leptin or its receptor mRNA levels were observed in mice treated with PEG-GLP-1/glucagon alone. Similarly, no difference in any mRNA measurements were observed in mice pretreated with vehicle and subsequently continued on PEG-leptin monotherapy, which is consistent with the lack of improvements in body weight or hormonal status in these mice. Notably, plasma levels of endogenous leptin, as well as mRNA levels of leptin and its receptor, showed a strong correlation with both body weight and body fat mass (Supplementary Fig. 2*A–F*), suggesting that the decrease in body fat mass accounts for the observed changes of leptin and its receptor.

Metabolic Effects of Leptin Monotherapy After Terminating Adjunctive Therapy With GLP-1/Glucagon

To assess whether PEG-leptin sustains the improved metabolic benefits after adjunctive GLP-1/glucagon

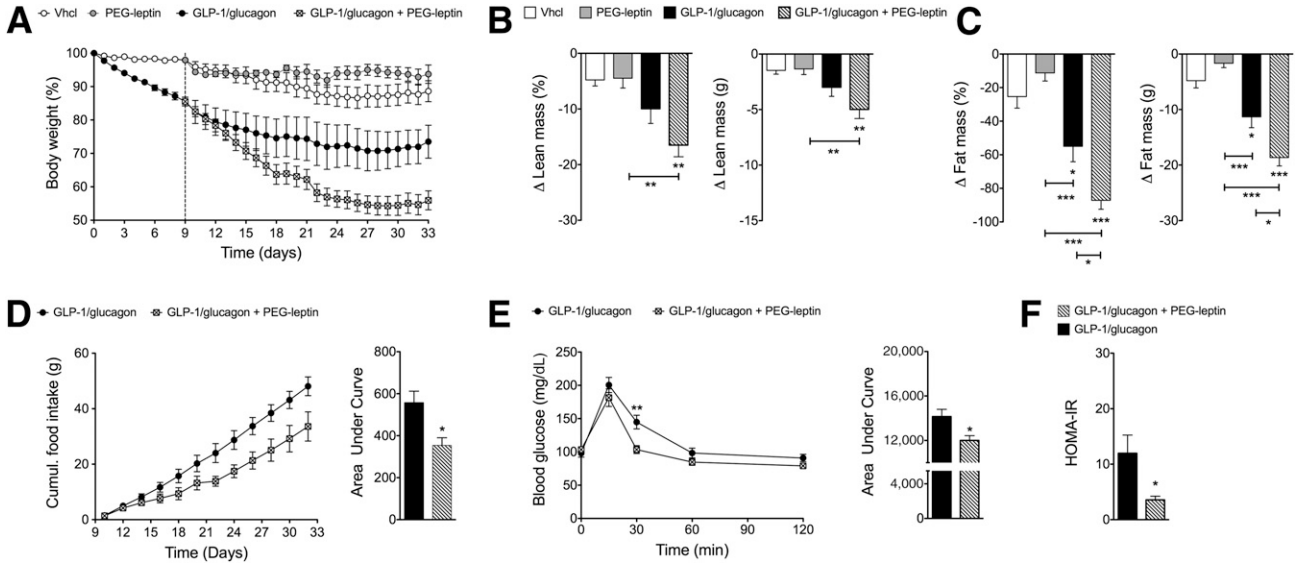

Figure 2— Effect of adjunctive treatment of DIO mice with PEG-GLP-1/glucagon and PEG-leptin following initial body weight loss induced by PEG-GLP-1/glucagon monotherapy. (*A*) Percentage body weight, (*B* and *C*) change of body composition, (*D*) cumulative food intake, (*E*) glucose tolerance, and (*F*) homeostasis model assessment of insulin resistance of DIO mice treated chronically (subcutaneous) from days 9 to 33 with either vehicle (n = 7), PEG-leptin (3 mg/kg/day; n = 7), PEG-GLP-1/glucagon (30 nmol/kg/4 days; n = 6), or the combination of PEG-GLP-1/glucagon and PEG-leptin (n = 15). Injections of vehicle and PEG-leptin were given daily, injections of PEG-GLP-1/glucagon were given at study days 12, 16, 20, 24, 28, and 32. (*B* and *C*) Changes in body composition reflect changes from day 0 to 32. Glucose tolerance (intraperitoneal; 1.5 g glucose/kg body weight) was assessed at study day 32. The dashed line at study day 9 indicates start of the PEG-leptin–PEG-GLP-1/glucagon combination therapy. Data represent means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001; Vhcl, vehicle; Cumul., cumulative; HOMA-IR, homeostasis model assessment of insulin resistance.

therapy, a subset of mice cotreated with PEG-GLP-1/glucagon and PEG-leptin were continued on PEG-leptin alone starting on study day 21. At day 33, mice continued on PEG-leptin alone showed no statistical difference in body weight relative to mice treated with the combination therapy but showed a trend of increased weight gain (Fig. 3*A*). However, mice that were treated with PEG-leptin alone showed an increased lean tissue mass ($P <$ 0.01) without any difference in body fat mass or food intake as compared with mice continued on the combination therapy (Fig. 3*B–D*). Mice randomized to PEG-leptin monotherapy were unable to sustain the improved glucose tolerance and insulin sensitivity observed by adjunctive administration with PEG-GLP-1/glucagon (Fig. 3*E* and *F*), corroborating the profound effect of the coagonist on glucose metabolism. Despite no overall changes in body weight, fat mass, and food intake, levels of liver triglycerides and plasma levels of insulin, cholesterol, and endogenous leptin all increased in mice continued on PEG-leptin monotherapy relative to mice continued on the combination therapy (Supplementary Fig. 1*A–D*). Collectively, these data suggest that leptin monotherapy is incapable of sustaining the improved metabolic state once GLP-1/glucagon is removed. Our observations, however, warrant further studies over a longer time period as a means to increase success in translation to clinical studies. Of specific interest is the change in body composition during leptin monotherapy following combination therapy.

DISCUSSION

Leptin resistance impairs the efficacy of leptin pharmacology when administered as stand-alone therapy. Treatment with amylin, exendin-4, or FGF21 was shown to restore leptin responsiveness and when combined with leptin synergistically lowers body weight in obese rodents (9,10). These observations encourage further consideration of leptin-based polypharmacy. A central limitation of these studies, however, is that return of leptin action required a moderately low content of dietary lipids. The observation that coadministration of PEG-leptin with exendin-4 or FGF21 failed to restore leptin responsiveness when mice were maintained on a HFD (10) correlates with recent reports demonstrating that leptin resistance occurs immediately following HFD exposure and prior to an increase in adiposity (14,18,19,22,23). These findings collectively demonstrate that a HFD is a detrimental factor contributing to the development of leptin resistance and underscore the importance of addressing the obesogenic environment to unleash the beneficial aspects inherent to leptin therapy.

PEG-GLP-1/glucagon coagonism reverses obesity and the associated metabolic syndrome in rodents maintained on a HFD (20). We report here that treatment with the same PEG-GLP-1/glucagon coagonist restores leptin responsiveness in mice chronically exposed to a HFD. Coadministration of PEG-GLP-1/glucagon and PEG-leptin resulted in ~18% greater reduction in body weight as compared with PEG-GLP-1/glucagon alone,

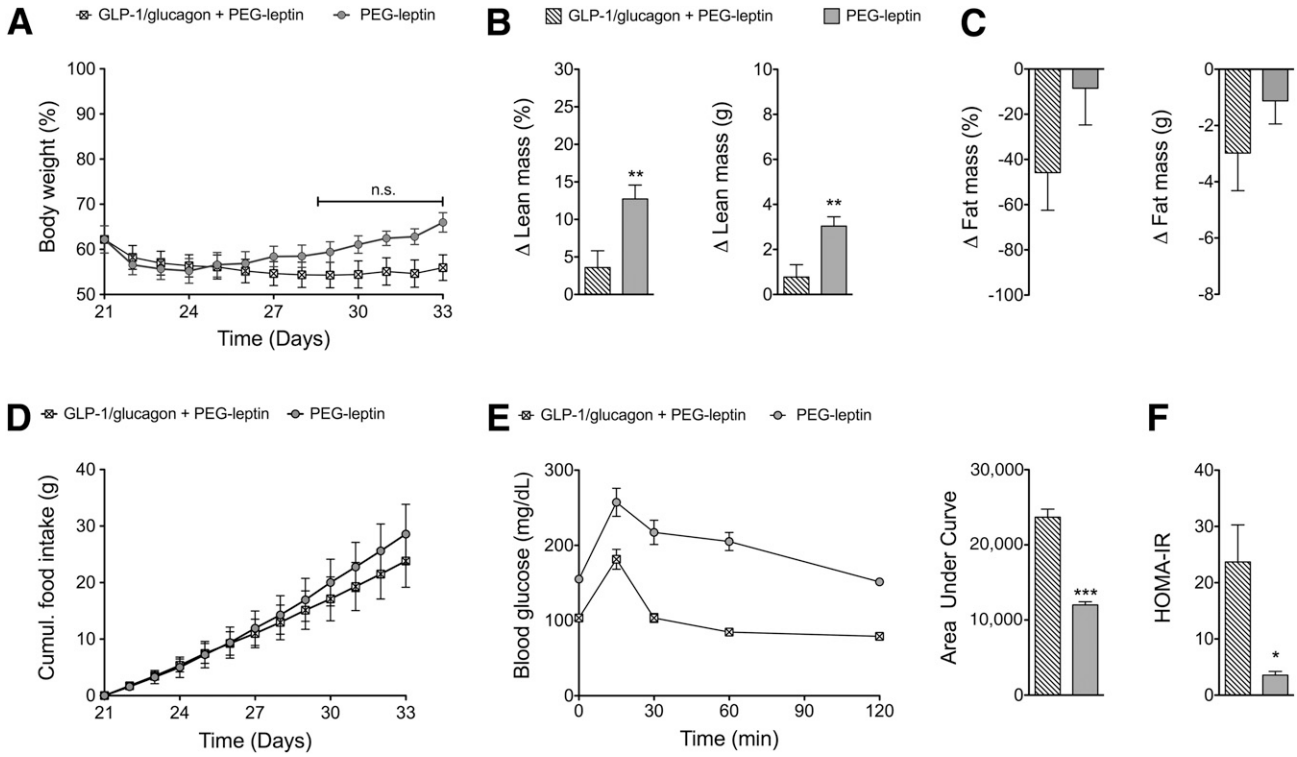

Figure 3—Effect of PEG-leptin monotherapy following pretreatment with PEG-GLP-1/glucagon and PEG-leptin. (*A*) Percentage body weight, (*B* and *C*) change of body composition, (*D*) cumulative food intake, (*E*) glucose tolerance, and (*F*) homeostasis model assessment of insulin resistance of DIO mice treated chronically (subcutaneous) from days 9 to 21 with PEG-GLP-1/glucagon and PEG-leptin and that have then been continued on either the combination of PEG-leptin and PEG-GLP-1/glucagon (n = 6) or PEG-leptin alone (3 mg/kg/day; n = 7). Injections of PEG-leptin were given daily, injections of PEG-GLP-1/glucagon was given at study days 24, 28, and 32. (*A*) Percentage changes in body weight reflect changes from days 0 to 33. (*B* and *C*) Changes in body composition reflect changes from days 20 to 32. Glucose tolerance (intraperitoneal; 1.5 g glucose/kg body weight) was assessed at study day 32. Data represent means ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; n.s., not significant; Cumul., cumulative; HOMA-IR, homeostasis model assessment of insulin resistance.

whereas leptin monotherapy without prior PEG-GLP-1/glucagon treatment had no effect on body weight. The beneficial effect of cotherapy with PEG-leptin was associated with decreased food intake, improved glucose tolerance and insulin sensitivity, decreased liver triglycerides, and lower plasma levels of insulin and cholesterol. The pronounced weight loss observed by PEG-GLP/glucagon and PEG-leptin cotherapy was associated with reduction of endogenous, circulating leptin that was paralleled by a substantial decrease in leptin mRNA levels in epididymal white adipose tissue and hypothalamic leptin receptor expression. The alteration in leptin homeostasis mirrors the marked decrease in adipose tissue as mRNA levels of leptin and its receptor strongly correlate with the amount of body fat mass. Hypothalamic expression of *Npy* and *Agrp* were increased in these mice as compared with vehicle controls, potentially reflecting the negative energy balance caused by the combination therapy.

Of particular importance are the observations made following the discontinuation of combination therapy. In the group randomized to PEG-leptin monotherapy following cotreatment with PEG-GLP-1/glucagon, we observed a trend for regaining body weight that was associated with an increase in lean but not fat tissue mass. Despite no change in body weight or fat mass, these mice showed increased plasma levels of leptin, insulin, cholesterol, and liver triglycerides as well as a reduction in hypothalamic expression of *Npy* and *Agrp* following removal of PEG-GLP-1/glucagon. These biochemical measures indicate that leptin monotherapy following successful cotherapy with PEG-GLP-1/glucagon is failing to maintain the beneficial effects on glucose and lipid metabolism in the sustained obesogenic environment.

In summary, a series of evidence indicates that the abundance of dietary fat and sugar contribute to the development of leptin resistance and constitute a major impediment to successful treatment of obesity. Consequently, our results are of sizable, potential therapeutic importance, as GLP-1/glucagon coagonism restores leptin responsiveness in obese mice without dietary change, a dietary setup where exendin-4 and FGF21 failed to improve leptin responsiveness. Ideally, lifestyle modifications should be used as central tools when it comes to lowering excessive weight and preventing the progression of obesity. Nonetheless, the epidemic nature of the disease and its heterogeneity requires diverse and

complementary approaches. Identification of a means to unlock the beneficial effects that endogenously reside in leptin but are suppressed in conventional forms of obesity remains one of the more promising approaches to successfully address the obesity epidemic. These results advance the prospect that leptin pharmacology in combination with other medicinal agents, most notably GLP-1/glucagon coagonists, might be effective in a clinical setting associated with less intensive lifestyle modification, rendering it more suitable to a broader population requiring therapy.

Acknowledgments. The authors thank Carola Meyer (Institute for Diabetes and Obesity, Helmholtz Center Munich) for her skillful assistance with obtaining animal ethics approval and Joe Tuchsherer (Ambrx) for assistance in chromatography related to leptin production.

Funding. This work was supported by grants from the Deutsche Forschungsgemeinschaft (TS226/1-1) and the Alfred Benzon Foundation.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. C.C. performed the experiments, evaluated the data, and drafted the manuscript. J.C. and L.Su. made substantial contributions in compound development, evaluated the data, and helped draft the manuscript. B.F., K.F., D.K., and L.Se. helped with the in vivo experiments, evaluated the data, and helped draft the manuscript. T.O. and S.M.H. performed the lipid profiling and revised the manuscript critically. S.C.S. and P.T.P. helped with the in vivo experiments and revised the manuscript critically. J.P. and R.D. made substantial contributions in compound development, made substantial contributions in the study design and interpretation of data, and helped edit the manuscript. M.H.T. made substantial contributions in the study design and interpretation of data and helped edit the manuscript. T.D.M. helped with the in vivo experiments, evaluated the data, helped draft the manuscript, made substantial contributions in the study design and interpretation of data, and helped edit the manuscript. T.D.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. 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
2. Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci U S A 1997;94:8878–8883
3. Halaas JL, Gajiwala KS, Maffei M, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995;269:543–546
4. Licinio J, Caglayan S, Ozata M, et al. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. Proc Natl Acad Sci U S A 2004;101:4531–4536
5. Farooqi IS, Matarese G, Lord GM, et al. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest 2002;110:1093–1103
6. Heymsfield SB, Greenberg AS, Fujioka K, et al. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. JAMA 1999;282:1568–1575
7. Scarpace PJ, Zhang Y. Leptin resistance: a prediposing factor for diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 2009;296:R493–R500
8. Hukshorn CJ, Saris WH, Westerterp-Plantenga MS, Farid AR, Smith FJ, Campfield LA. Weekly subcutaneous pegylated recombinant native human leptin (PEG-OB) administration in obese men. J Clin Endocrinol Metab 2000;85:4003–4009
9. 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 U S A 2008;105:7257–7262
10. Müller TD, Sullivan LM, Habegger K, et al. Restoration of leptin responsiveness in diet-induced obese mice using an optimized leptin analog in combination with exendin-4 or FGF21. J Pept Sci 2012;18:383–393
11. Trevaskis JL, Lei C, Koda JE, Weyer C, Parkes DG, Roth JD. Interaction of leptin and amylin in the long-term maintenance of weight loss in diet-induced obese rats. Obesity (Silver Spring) 2010;18:21–26
12. Sadry SA, Drucker DJ. Emerging combinatorial hormone therapies for the treatment of obesity and T2DM. Nat Rev Endocrinol 2013;9:425–433
13. Coppari R, Bjørbæk C. Leptin revisited: its mechanism of action and potential for treating diabetes. Nat Rev Drug Discov 2012;11:692–708
14. Vasselli JR, Scarpace PJ, Harris RB, Banks WA. Dietary components in the development of leptin resistance. Adv Nutr 2013;4:164–175
15. Vasselli JR. The role of dietary components in leptin resistance. Adv Nutr 2012;3:736–738
16. Vasselli JR. Fructose-induced leptin resistance: discovery of an unsuspected form of the phenomenon and its significance. Focus on "Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding," by Shapiro et al. Am J Physiol Regul Integr Comp Physiol 2008;295:R1365–R1369
17. Vasselli JR. Behavioral and biological determinants of leptin resistance. Appetite 2001;37:115–117
18. Thaler JP, Yi CX, Schur EA, et al. Obesity is associated with hypothalamic injury in rodents and humans. J Clin Invest 2012;122:153–162
19. Wang J, Obici S, Morgan K, Barzilai N, Feng Z, Rossetti L. Overfeeding rapidly induces leptin and insulin resistance. Diabetes 2001;50:2786–2791
20. 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
21. Beiroa D, Romero-Picó A, Langa C, et al. Heterozygous deficiency of endoglin decreases insulin and hepatic triglyceride levels during high fat diet. PLoS ONE 2013;8:e54591
22. Banks WA, Coon AB, Robinson SM, et al. Triglycerides induce leptin resistance at the blood-brain barrier. Diabetes 2004;53:1253–1260
23. Shapiro A, Mu W, Roncal C, Cheng KY, Johnson RJ, Scarpace PJ. Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high-fat feeding. Am J Physiol Regul Integr Comp Physiol 2008;295:R1370–R1375