

Pas de Deux

Glucagon and Thyroid Hormone Moving in Perfect Synchrony

Alexandra L. Ghaben, Philipp E. Scherer

Chemical hybridization of glucagon and thyroid hormone optimizes therapeutic impact for metabolic disease

Finan et al

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The implementation of thyroid hormone (T3) or glucagon as lipid-lowering, antidiabetic, and antiobesity agents have not been successful to date because of serious adverse side effects. Using a synthetic hybrid glucagon/T3 conjugate, Finan et al have established a novel therapeutic approach for the reversal of dyslipidemia, obesity, and insulin resistance associated with the metabolic syndrome, while limiting cardiovascular side effects.

Obesity-associated dyslipidemia is the primary risk factor for cardiovascular and other metabolic diseases.¹ Increased circulating lipid levels contribute to atherosclerosis and coronary artery disease,² whereas increased lipid deposition in tissues, such as adipose tissue, skeletal muscle, and liver, increases the risk of insulin resistance.³ Statins have been a mainstay of dyslipidemia treatment since their introduction in the 1980s. Statins reduce cardiovascular disease incidence through a reduction of circulating low-density lipoprotein and reduced atherosclerotic lesion formation, but minimally address other lipid-related species (triglycerides, high-density lipoprotein) and ectopic accumulation of tissue lipids.⁴

In a recent article published in *Cell*, Finan et al⁵ report a novel pharmacological tool with impressive metabolic benefits. The backbone consists of 2 well-established hormones: glucagon and thyroid hormone. These 2 entities are chemically conjugated and the authors refer to it as glucagon/T3. This hybrid molecule corrects multiple aspects of obesity-associated dyslipidemia that are not addressed by current therapeutics. With relatively short (2- to 3-week) administrations of glucagon/T3, the authors effectively managed to reduce low-density lipoprotein-cholesterol, reverse steatohepatitis, prevent atherosclerotic plaque accumulation, improve glucose

tolerance, and reduce body weight in a variety of rodent models of obesity. Exposure to this novel compound has broad physiological implications: First, it provides a method to leverage 2 pathways for the treatment of metabolic disease that have to date not been successfully tapped into individually. Second, it provides a new entity with differential pharmacodynamic properties distinct from the 2 parent molecules (Figure).

Thyroid Hormone and Glucagon, Therapeutic Promise With Challenges

Individually, thyroid hormone and glucagon are imperfect but promising candidates for metabolic therapeutics. Clinically, overt hypothyroidism is associated with increased weight, increased adiposity, increased circulating low-density lipoprotein, and risk for atherosclerosis, as well as a decreased metabolic rate.^{6,7} Somewhat paradoxically, an elevation of thyroid hormone and thyroid-stimulating hormone is observed in obese individuals with normal thyroid function, leading to the concept of peripheral thyroid resistance in obesity, mediated through decreased levels of thyroid receptor in adipocytes.⁸ Despite the favorable metabolic effects of thyroid hormone, the stimulation of thyroid receptor in euthyroid individuals as a means of reducing obesity has not met success. Systemic thyroid receptor agonism has led to serious adverse effects, including increased heart rate, cardiac hypertrophy, muscle wasting, and decreased bone density. To combat these effects, thyroid hormone receptor β -selective agonists have been developed to target the effects of thyroid hormone to the liver and improve the systemic lipid profile. These agents have shown varying degrees of clinical promise, but none have successfully completed a phase III clinical trial without significant side effects (including elevated serum transaminase levels and increased bone turnover).⁹ Notably, even the most successful implementation of these liver-specific thyroid receptor agonists is unlikely to improve metabolism in other key metabolic targets, such as adipose tissue and skeletal muscle.

Glucagon is largely appreciated for its antihypoglycemic counterregulatory effects to insulin. However, glucagon is characteristically elevated in the diabetic state, and multiple therapeutic attempts have been made to inhibit glucagon signaling (through glucagon-like peptide 1 agonism or dipeptidyl peptidase-IV inhibitors) or to antagonize it (with antiglucagon receptor antibodies or glucagon receptor antagonists). These approaches have frequently proven to be effective at the pre-clinical, as well as at the clinical, level for improvements in glycemic control. However, several issues have arisen with these approaches. Interfering with the glucagon signaling axis may lead to increased serum cholesterol, increases in body weight, changes in glycogen storage, pancreatic α -cell hyperplasia, and increases in serum transaminase levels. An additional concern is the potential for hypoglycemia.¹⁰ The

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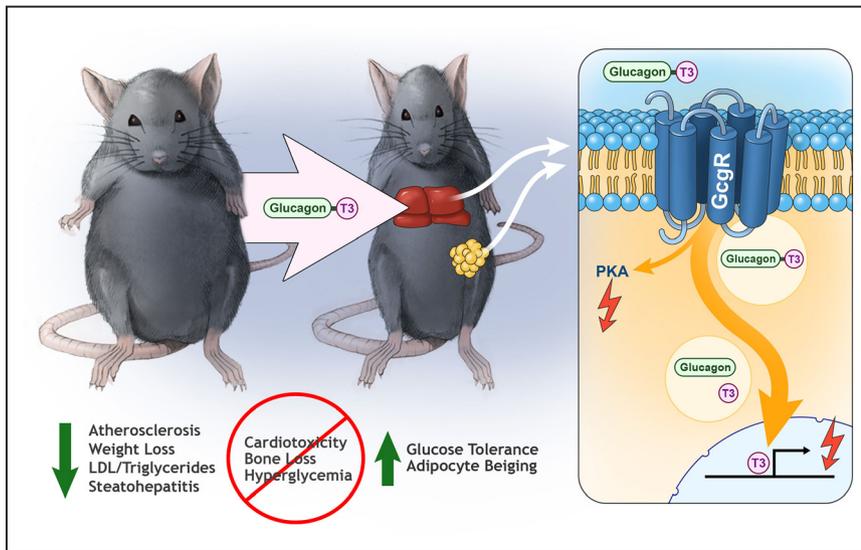


Figure. Systemic effects of the glucagon/T3 hybrid molecule. A novel method to unlock both the beneficial antilipid effects of glucagon and the energy-expending effects of thyroid hormone by chemically conjugating the 2 entities. The molecule is thought to act mostly in the liver and in adipocytes, with glucagon acting as the primary component to deliver the T3 to liver and adipose tissue. This hybrid moiety bypasses many of the established side effects of the individual molecules and has potent glucose-lowering and antilipidemic effects. The glucagon/T3 hybrid gets intracellularly cleaved in lysosomes (circular structures). LDL indicates low-density lipoprotein; and PKA, protein kinase A activation. Figure created by Richard Howdy, certified medical illustrator, at Visually Medical (<http://www.visuallymedical.com>).

approach remains, however, viable and has shown great promise in some recent studies.¹¹

Glucagon/T3 Hybrid

The new hybrid molecule described by Finan et al⁵ displays remarkable lipid-lowering and antihyperglycemic effects. The underlying mechanisms by which this hybrid glucagon/T3 achieves these remarkable physiological responses are partially solved. The reductions in serum lipids are mediated at least in part by contributions from the liver and require the presence of both hormone components. Mice congenitally lacking the glucagon receptor fail to increase T3 concentrations in the liver on glucagon/T3 treatment and, thus, do not exhibit any reductions in cholesterol or triglyceride. Mice carrying a liver-specific deletion of the thyroid hormone receptor also fail to reduce cholesterol or triglyceride after treatment. Taken together, the authors suggest a model in which the glucagon component of glucagon/T3 compound mediates targeting to the proper tissues, and the observed lipid improvements are primarily driven through thyroid hormone receptor- β signaling. This synergistic interaction of glucagon and T3 signaling is confirmed when examining transcriptional changes in livers from the glucagon/T3-treated mice. The transcriptional enhancements seen support the idea that the synergistic action of the hybrid compound yields enhanced activation of the signaling pathways and thereby contributes to greater improvements than either compound alone or coadministered without a chemical link.

Importantly, the serum lipid improvements obtained with glucagon/T3 treatment are paralleled by improvements in tissue lipid handling. Three weeks of treatment with glucagon/T3 of mice with fatty liver and an early indication of nonalcoholic steatohepatitis result in reduced lipid accumulation, reduced body weight along with a lowering of alanine transaminase levels. Notably, the mice treated with glucagon/T3 also demonstrate a reduction in blood glucose.

In addition, glucagon/T3 improves adipose tissue function through induction of beiging and increasing energy expenditure. This effect seems to be mediated primarily through an increased metabolic rate in tissues because the mice display

no change in food intake or activity. One way that glucagon/T3 may increase the metabolic rate is through beiging of white adipose. Beiging of white adipose tissue is characterized by a switch from unilocular white adipocytes to multilocular beige adipocytes with increased numbers of mitochondria, increased and partially uncoupled respiration. The glucagon/T3-induced beiging of white adipose tissue and the induction of the thermogenic program occur to a similar extent than with T3 alone. However, the glucagon/T3 compound provides a more targeted weight loss at the systemic level. Unlike T3, the beiging of white adipose by glucagon/T3 is not offset by an increase in food intake, so the increased metabolic rate is more directly translated to weight loss. This may be because of the targeting of the glucagon/T3 conjugate specifically to adipose tissue via the glucagon receptor, thereby avoiding off-target actions in tissues, such as the brain. Moreover, the safety profile of the beiging induced by glucagon/T3 is more favorable than that achieved with T3 monotherapy. T3 induces an elevation of core temperature above baseline, whereas glucagon/T3 does not. It is important to note that the beiging of white adipose is not the only mechanisms by which glucagon/T3 increases energy expenditure because uncoupling protein 1 (UCP1) null mice still display an increase in energy expenditure (albeit blunted). We do not know much about the nature of these UCP1-independent mechanisms of increased energy expenditure, but the authors suggest fibroblast growth factor 21 (FGF21) as a possible driving force.

The most surprising aspect of this new glucagon/T3 hybrid is the ability to confer key beneficial metabolic effects of each constituent, while offsetting the negative side effects observed for each agonist separately. Glucagon/T3 does not display the diabetogenic properties of glucagon; in fact, it even improves glucose tolerance and reduces plasma insulin levels. This effect is specific to the hybrid glucagon/T3 molecule, as coadministration of unconjugated glucagon and T3 does not prevent glucagon-induced increases in hepatic gluconeogenesis. Molecularly, conjugated T3 seems to offset the gluconeogenic effects of glucagon through simultaneous induction of the glycolytic program. This creates a state where glucagon signaling proximal to PKA (protein kinase A activation) and

PGC1 α (PPAR γ coactivator 1 α) activation is intact, but the concomitant actions of T3 in the conjugated compound are able to blunt activation of CREB and PGC1 β , thereby reducing the overall gluconeogenic stimulus of glucagon/T3 in the liver compared with glucagon alone. Moreover, mice treated with glucagon/T3 exhibit none of the cardiotoxicity observed with long-term thyroid hormone supplementation. No reduction in bone volume was seen in the glucagon/T3 group, whereas mice treated with T3 alone lost \approx 25% of their pre-treatment bone volume. Promising as these preclinical safety profiles are, additional research with longer dosing regimens will be required before taking the compound to the clinic.

Clinical/Translational Implications

In the preclinical models, the use of glucagon/T3 in obese, metabolically dysfunctional mice corrects serum dyslipidemia, reverses nonalcoholic steatohepatitis, reduces atherosclerotic plaque accumulation, improves glucose metabolism, and reduces adipose mass while avoiding thyrotoxicity and the diabetogenic effects of glucagon. The selective targeting of these hybrid molecules to discrete tissues may well be what sets the molecule apart from the actions achieved with the individual components. In addition, it is conceivable that the simultaneous activation of 2 signaling cascades in close physical proximity at the level of the plasma membrane creates a unique beneficial signaling microenvironment. However, although this may apply to bi- or triagonists that bind to cell surface receptors, this seems to be less likely in the context of this glucagon/T3 hybrid that represents a combination of cell surface receptor activation with nuclear receptor signaling.

Finally, the devil may lie in the details with respect to the leap from the preclinical to the clinical setting. There is a much stronger hepatic gluconeogenic component to the rodent models of metabolic dysfunction compared with humans. Furthermore, if the preclinical phenotype is taking advantage of the effects driven by the adipocyte, this may be harder to achieve in humans because of the comparatively low levels of expression of the glucagon receptor in adipocytes, as well as reduced capacity of adult human adipocytes to undergo beiging. It is obviously premature to speculate on the potential of this compound for clinical efficacy. Nevertheless, the authors have made a great conceptual leap in the use of this combinatorial

chemistry to leverage complimentary actions of 2 imperfect hormones to maximize benefits and minimize risks.

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Disclosures

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