CLINICAL IMPLICATIONS OF BASIC RESEARCH

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Wasting Energy to Treat Obesity

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The concept of enzyme therapy dates back to at least the 19th century, when pancreatic enzymes were offered to patients who had exocrine pancreatic insufficiency.[1] In 1964, Christian de Duve suggested enzyme treatment as a therapeutic strategy for lysosomal disorder: three decades later, glucocerebrosidase replacement became the standard of treatment for Gaucher's disease.[2] Recently, Long et al.[3] described experiments that were designed to probe enzymatic activity that generates a class of amino acids capable of short-circuiting the flow of mitochondrial energy, akin to but independent of the uncoupling protein 1 (UCP1) of brown adipose tissue. Mice that are treated with the enzyme or its metabolites lose weight as a result of uncoupled "energy wastage" in cells other than brown adipocytes. These findings raise the possibility of fighting obesity through supplementation with this enzyme or its biochemical products.

Creating a negative energy balance to lose weight is easy in principle but difficult in practice in a sedentary society. Indeed, achieving a sustained increase in energy expenditure through regular exercise is extremely difficult for most if not all overweight or obese persons. Chemical uncoupling is therefore an attractive strategy for the dissipation of excess energy, because surplus calories are "wasted" (in the form of heat) through biochemically futile cycling of metabolic substrates without the need for physical mechanical effort. This strategy is not new: the pharmacologic uncoupler 2,4-dinitrophenol was widely used as a weight-loss agent during the previous century, but its use was discontinued because of its systemic toxic effects.[4]

In studies in rodents, Long and colleagues identified a previously unstudied enzyme, known as peptidase M20 domain–containing 1 (PM20D1), that has uncoupling and fat-burning actions. This enzyme meets some of the criteria for an anti-obesity enzyme therapy. First, it is secreted, detectable in plasma, and endogenously produced. Moreover, PM20D1 that is generated from a viral vector is resistant to degradation in the blood. Second, experimentally elevated levels of PM20D1 effect fat catabolism in animals, resulting in weight loss and glycemic improvement without compensatory orexigenic adaptation. Third, unlike uncoupling that is mediated by brown adipose tissue, which requires cold activation, PM20D1-induced weight loss occurs without cold exposure and therefore is suited to a thermoneutral environment (Fig. 1).

How does PM20D1 induce weight loss? Long et al. found that this enzyme stitches lipids onto amino acids to generate *N*-acyl amino acids. These newly synthesized lipidated metabolites turn on uncoupling in cells that lack UCP1, including white adipocytes and muscle cells. Although the underlying mechanisms await further elucidation, preliminary results have revealed binding of *N*-acyl amino acids to mitochondrial transporters (such as the SLC25 family), which may fuel proton shunting and thereby mimic UCP1 function. Remarkably, the treatment of mice with *N*-acyl amino acids increased energy expenditure and lowered glucose, thus recapitulating the metabolic benefits observed in mice that overexpress PM20D1. Therefore, the supplementation of these fat-burning *N*-acyl amino acids also represents a plausible experimental therapeutic strategy.

PM20D1 is not found in white fat cells and is found only in UCP1-expressing adipocytes. These include classic interscapular brown and beige adipocytes; the latter are "brown fat–like" cells that are induced within inguinal white fat after

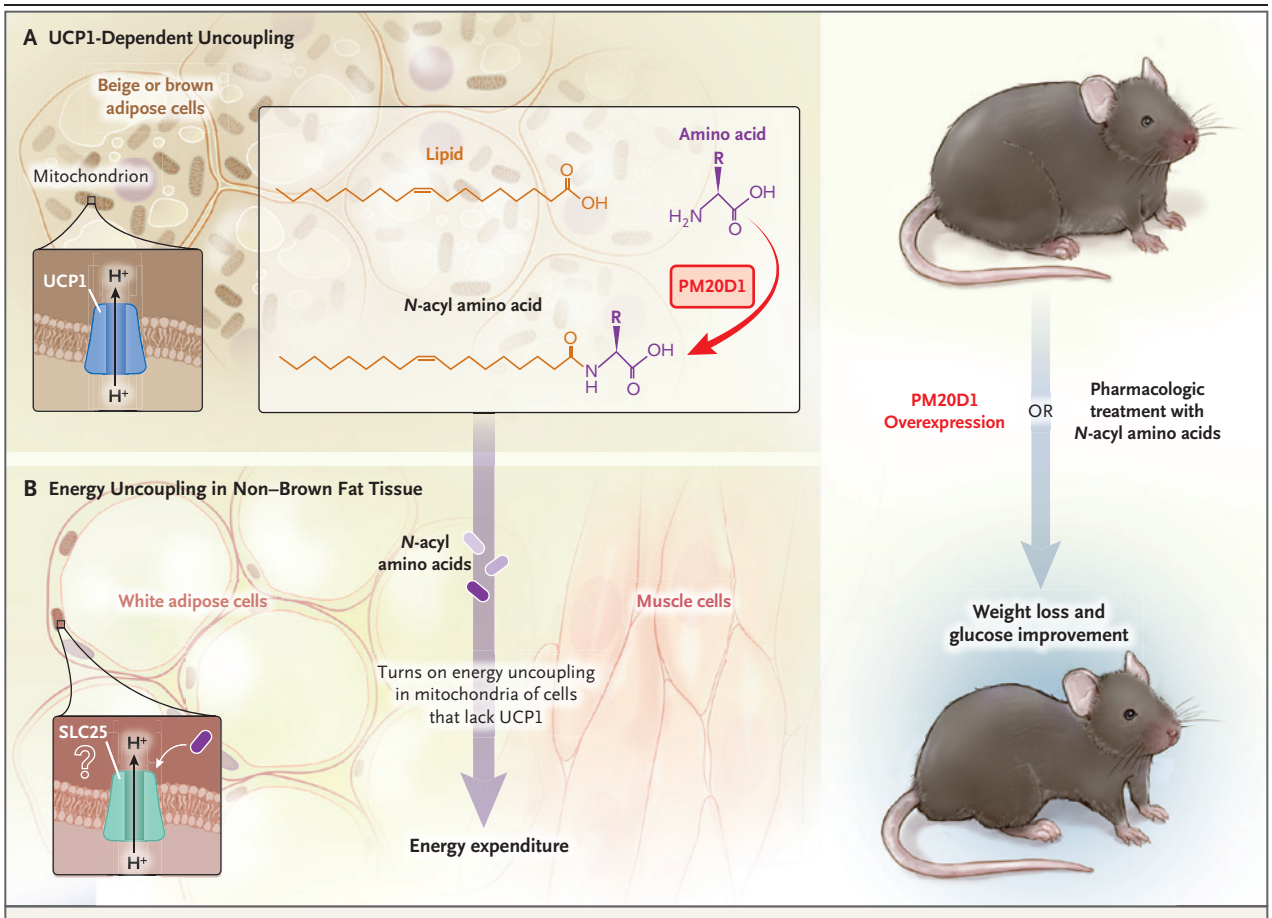

Figure 1. Wasting Energy to Lose Weight.

Chemical uncoupling occurs in mitochondria through proton shunting in an uncoupling protein 1 (UCP1)–dependent manner (UCP1 is unique to brown and beige fat[5]) and a UCP1-independent manner (such as with a pharmacologic uncoupler[4]). Induction of uncoupling leads to dissipation of excess energy and weight loss in mice. Long and colleagues[3] reported that peptidase M20 domain–containing 1 (PM20D1) is an enzyme that is enriched in classic brown and beige ("brown fat–like") adipocytes and secreted into the circulation and that catalyzes the synthesis of *N*-acyl amino acids (Panel A). Both the enzyme and its products are capable of triggering energy uncoupling in non–brown fat tissue, such as white fat and skeletal muscle, hypothetically by serving as ligands for mitochondrial SLC25 family carriers that act as proton translocators (Panel B). Overexpression of PM20D1 or pharmacologic treatment with *N*-acyl amino acids results in weight loss and improvement in glucose metabolism.

cold exposure. Although brown adipose tissue diminishes after infancy, a considerable abundance of adipocytes — which can be induced to become beige adipocytes — reside within the neck and subcutaneous white fat of adult humans.[5] The expression of PM20D1 in thermogenic UCP1-expressing adipocytes may be a hint regarding its evolutionary origin. On hypothermic threat, nonshivering (mediated by brown adipose tissue) and shivering (muscle-mediated) thermogenesis are progressively recruited to defend core temperature. Shivering disadvantages hunting and gathering efficiency and survival in the cold, and therefore selection may have favored the expression of an enzyme secreted by brown adipose tissue that ignites thermogenesis in other tissue types (such as white fat and muscle) — representing, as it were, an on-call hypothermia rescue corps — until inducible white adipocytes are "beiged" over time.

Furthermore, although plasma levels of PM20D1 are unchanged in cold-acclimated mice, cold exposure increases circulating levels of many *N*-acyl amino acids. From a diagnostic perspective, the PM20D1 enzyme–substrate–metabolite milieu may harbor a surrogate that is indicative of the

whole-body "brown–beige" reserve. A plasma "cold-exposed" *N*-acyl amino acid metabolomic signature may be exploitable as a trackable biomarker of thermogenic capacity in humans.

There are, of course, many issues to consider before PM20D1 or its products could be considered as candidates for antiobesity drugs. The physiological characteristics of PM20D1 need to be fully explored in humans, and the off-target toxic effects need to be scrutinized in preclinical models, as underscored by the fatalities caused by 2,4-dinitrophenol. However, the study by Long et al. has illuminated uncharted research territory in the antiobesity therapeutics arena and provides a welcome — albeit tentative — context for the many *N*-acyl amino acids that have been discovered in mammals and the biologic significance of which has remained largely unknown.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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1. BeitrageHEZur therapeutischen Verwendung der Bauchspeicheldruse von Schlachtthieren und deren Praparate. Dtsch Arch Klin Med 1879;24:539-82.
2. de Duve C. From cytases to lysosomes. Fed Proc 1964;23:1045-9.
3. Long JZ, Svensson KJ, Bateman LA, et al. The secreted enzyme PM20D1 regulates lipidated amino acid uncouplers of mitochondria. Cell 2016;166:424-35.
4. Tainter ML, Stockton AB, Cutting WC. Dinitrophenol in the treatment of obesity. JAMA 1935;105:332-6.
5. Kajimura S, Spiegelman BM, Seale P. Brown and beige fat: physiological roles beyond heat generation. Cell Metab 2015;22:546-59.

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