METABOLISM

The quest to burn fat, effortlessly and safely

An enzyme steps up to BAT as a potential mitochondrial uncoupler

By **Weiwei Fan** *and* **Ronald Evans**

Treatment of obesity and obesity-associated diseases has been challenging, with the first potential cure claimed in 1934 with the protonophore 2,4-dinitrophenol (DNP). This chemical dissipates mitochondrial membrane potential into heat production and is extremely effective in boosting metabolic rate and promoting weight loss (*1*). However, severe side effects, including cataract formation, cardiotoxicity, overheating, and death, prevented its further use (*2*). In a recent study, Long *et al.* (*3*) report that a secreted enzyme called peptidase M20 domain containing 1 (PM20D1) converts fatty acids and amino acids into *N*-acyl amino acids, which directly uncouple mitochondrial membrane potential in a way similar to that of DNP, to increase energy expenditure without physical movement. Might these endogenous metabolites be a safe alternative to chemical uncouplers, facilitating effortless fat burning without a fatal consequence?

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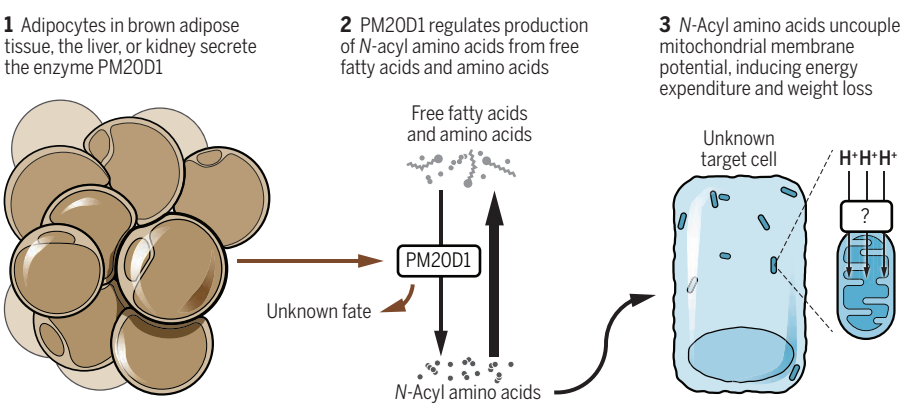
Mammals develop brown adipose tissue (BAT) and beige adipose tissue that are specialized in thermogenesis by their high expression of uncoupling protein 1 (UCP1), an endogenous mitochondrial uncoupler (*4*). To discover new proteins that contribute to thermogenesis, Long *et al.* conducted a combinatorial genomic and proteomic study and identified PM20D1, whose expression is highly enriched in UCP1-positive adipocytes. In mice, systemic delivery of PM20D1 (via an adeno-associated viral vector) boosted energy expenditure and reduced diet-induced obesity. Metabolomic and enzymologic analyses further revealed that PM20D1 catalyzes the conversion of fatty acids and amino acids into *N*-acyl amino acids, which directly uncouple mitochondrial membrane potential. When administered in mice, PM20D1 increased metabolic rate and promoted weight loss.

Although UCP1-mediated mitochondrial uncoupling in brown and beige fat is a critical component of heat production, other nonshivering thermogenic processes have been demonstrated in mice lacking UCP1 including the uncoupling of adenosine triphosphate (ATP) hydrolysis from Ca^{2+} transport by sarcolipin in muscle (*5*), and a compensatory creatine futile cycle in beige fat (*6*). The study of Long *et al.* suggests the possibility of an additional UCP1-independent thermogenic mechanism, but additional work is needed to elucidate the roles of PM20D1 and *N*-acyl amino acids. Although preferentially expressed in UCP1-positive adipocytes in brown and beige fat, the secreted enzyme PM20D1 and its products, *N*-acyl amino acids, have the potential to function in a paracrine or endocrine manner to promote heat production in other cells and tissues. Furthermore, PM20D1 is most highly expressed in liver and kidney, which are generally not considered to contribute to thermogenesis. Cold exposure induced the expression of many species of *N*-acyl amino acids in blood. However, the expression of PM20D1 in brown fat, as well as its circulating protein concentration, is not affected. This suggests the existence of other enzymes that catalyze the synthesis of *N*-acyl amino acids, which could also contribute to their elevation in mice that overexpress PM20D1. Indeed, the synthase activity of PM20D1 is much lower than its hydrolase activity, which is consistent with the previously reported hydrolase activities in other PM20D1 members, including aminoacylase, carboxypeptidase, dipeptidase, and aminopeptidase activities (*7*). More important, although the overexpression of PM20D1 increases the plasma concentration of *N*-acyl amino acids, it is not clear whether this rise directly contributes to energy expenditure and thermogenesis, because their physiological amounts are much lower than what is required to stimulate mitochondrial uncoupling. Therefore, whether the weight-loss phenotype observed in PM20D1-overexpressing mice is indeed mediated by *N*-acyl amino acids or by other targets or products of PM20D1 needs further exploration. In addition, although Long *et al.* suggest a paracrine mechanism for PM20D1 and *N*-acyl amino acid-induced thermogenesis in brown and beige fat, this idea requires further demonstration that physiological concentrations of PM20D1 and *N*-acyl amino acids within these tissues are high enough to affect mitochondrial membrane potential.

The study of Long *et al.* reawakens the

Mitochondrial uncoupling and thermogenesis

N-Acyl amino acids are generated from free fatty acids and amino acids by the enzyme PM20D1, which is secreted by adipocytes in fat tissue. The *N*-acyl amino acids act as mitochondrial uncouplers, thereby boosting energy expenditure, but it is not yet clear in which tissues this occurs.



GRAPHIC: V. ALTOUNIAN/*SCIENCE*

promise of chemical-based mitochondrial uncoupling as a therapeutic strategy for obesity and obesity-associated diseases. Recent progress has been made toward developing liver-specific DNP derivatives or milder mitochondrial uncouplers that are effective in treating obesity and obesity-associated diseases but with minimal side effects (*8*–*10*). The identification of *N*-acyl amino acids as endogenous mitochondrial uncouplers would not only advance our understanding of adaptive thermogenesis, but also might present safer alternatives to chemical uncouplers if a direct role of *N*-acyl amino acids as mitochondrial uncouplers can be established. This may require the development of *N*-acyl amino acid mimetics, given the higher hydrolase over synthase activity of PM20D1. An immediate question that should be addressed is that in cell culture, *N*-acyl amino acids take 20 to 40 min to initiate uncoupling, which is much longer than the time taken by known chemical uncouplers such as DNP. In addition, *N*-acyl amino acids such as *N*-arachidonyl glycine (C20:4-Gly) have a wide range of biological functions via their interactions with G protein–coupled receptors and ion channels in brain and other tissues (*11*), which in vivo could contribute appreciably to the food suppression and weight-loss phenotypes observed in the treated mice. Furthermore, because the uncoupling effect of *N*-acyl amino acids is UCP1-independent and thus not limited to brown and beige fat, their role in mitochondrial ATP production in highly energetic tissues such as heart, brain, and kidney needs to be explored.

The findings of Long *et al.* open a door on a new class of endogenous mitochondrial uncouplers and present a new mechanism of adaptive thermogenesis via a secreted enzyme and its products. However, every open door reveals more questions than it answers, and follow-up studies are required. We are left to ponder the hope of a magic pill offering effortless and consequence-free fat burning. ■

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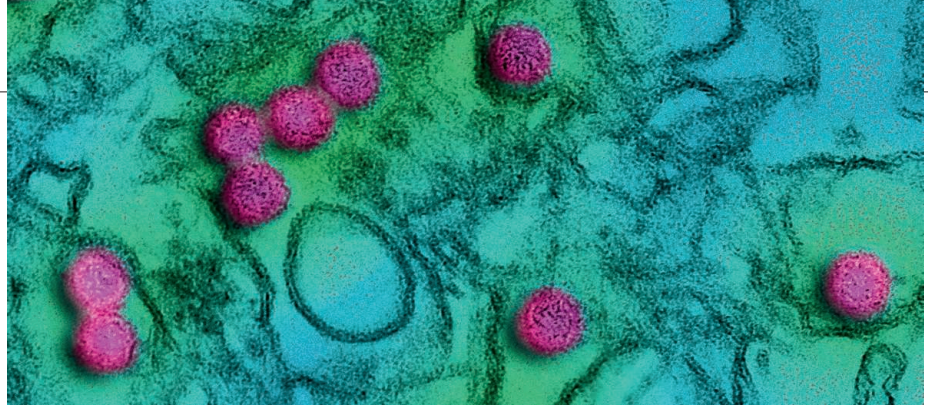

Well-characterized antibodies to ZIKV (pink; transmission electron micrograph shown) are needed.

VIROLOGY

Diagnostics for Zika virus on the horizon

The immune response to Zika virus informs antibody-based diagnostics and therapeutics

By **Scott D. Speer** *and* **Theodore C. Pierson**

Zika virus (ZIKV) is a mosquito-transmitted flavivirus that is related to other pathogens of clinical importance, including yellow fever and dengue (DENV) viruses. Although once infrequently associated with human disease, ZIKV has emerged as a global health threat with its introduction into South America during 2014 and 2015. Of concern, recent ZIKV outbreaks are linked to severe neurodevelopmental complications in the children of women infected while pregnant, as well as Guillain-Barré syndrome in adults (*1*). Management of this epidemic has been complicated by extensive serological cross-reactivity among flaviviruses and the cocirculation of ZIKV and DENV in regions experiencing the greatest disease burden. Current serological diagnostics have a limited capacity to distinguish between DENV and ZIKV. On page 823 of this issue, Stettler *et al.* (*2*) characterize monoclonal antibodies (mAbs) isolated from ZIKV-infected humans that hold promise as diagnostics or therapeutics, and advance our understanding of the repertoire of antibodies elicited by ZIKV infection.

Flaviviruses are assembled from three viral structural proteins [capsid, premembrane (prM), and envelope (E)], a host-derived lipid envelope, and the genomic viral RNA (*3*). Flavivirus-infected cells also secrete a nonstructural protein 1 (NS1), which has multiple roles in viral replication and pathogenesis in vivo (*4*). Both NS1 and the structural proteins are immunogenic. Virus-neutralizing antibodies most commonly target the E protein, may be highly protective in vivo, and are a correlate of protection for many flavivirus vaccines (*5*). NS1 antibodies are non-neutralizing, yet they contribute to protection via antibody heavy chain–mediated effector functions (*6*). Whereas the functional characteristics of antibodies in ZIKV-immune individuals have been studied (*7*), human ZIKV mAbs have not been reported.

Accordingly, Stettler *et al.* have now isolated a panel of 119 human mAbs from the memory B cells of four ZIKV-infected donors; two of these subjects had been infected previously by DENV (*2*). Roughly two-thirds of the mAbs produced bound epitopes within the E protein. Antibodies specific for the immunoglobulin (Ig)–like domain III (DIII) had considerable neutralizing activity and were largely specific for either ZIKV or DENV. Numerous cross-reactive mAbs with modest neutralization capacity mapped to E protein domains I or II. Domain II is the location of the highly conserved fusion loop frequently targeted by antibodies elicited by other flaviviruses. Although more study is required, cross-reactive fusion loop–specific antibodies may also be common in ZIKV-immune individuals. Of considerable interest, Stettler *et al.* found that the most potent neutralizing mAbs bound efficiently to intact virions but not to soluble forms of the E protein, which suggests that antibodies that bind quaternary epitopes composed of more than a single ZIKV E protein may be desirable. In agreement, three recent studies detail the recognition and functional properties of neutralizing mAbs that bind a quaternary epitope shared by ZIKV and DENV (*8*–*10*). E protein antigens

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