

# Lipolysis: more than just a lipase

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**Successful adaptation to starvation in mammals depends heavily on the regulated mobilization of fatty acids from triacylglycerols stored in adipose tissue. Although it has long been recognized that cyclic AMP represents the critical second messenger and hormone-sensitive lipase (HSL)\* the rate-determining enzyme for lipolysis, simple activation of the enzyme has failed to account for the robust augmentation of fatty release in response to physiological agonists. In this issue, Sztalryd et al. (2003) provide convincing support to the notion that the subcellular compartmentalization of lipase also regulates lipolysis, and, more importantly, that proteins other than HSL are localized to the lipid droplet and are indispensable for its optimal hydrolysis.**

Triacylglycerol (TAG) exists as the most efficient macromolecule for the storage of calories for times of fasting or increased energy demand. In recent years, however, researchers have come to appreciate not only complexity in the regulation of lipid mobilization and metabolism, but its importance to processes other than the supply of nutrients during intervals of meager food consumption or increased exercise. For example, TAG lipolysis produces glycerol and fatty acids. The resulting elevated circulating fatty acids are an exceptional candidate for an etiological factor in Type 2 diabetes mellitus, by virtue of their ability to promote insulin resistance in skeletal muscle. In spite of its importance, the cell biology of adipocyte lipid mobilization has received remarkably little attention, especially considering the abundance of research devoted to understanding the mobilization of glycogen, another repository for energy. As an illustration of this discrepancy, it is interesting to note that, even though Edwin Krebs published a seminal paper demonstrating the enhancement of lipolysis and neutral lipase activity by exposure to PKA in the presence of cyclic AMP, his Nobel Prize lecture does not make mention of TAG hydrolysis (Corbin et al., 1970; Krebs, 1993).

What then accounts for this seeming slur on lipid metabolism? It is, of course, difficult to be certain, although at least

two factors are likely to have contributed: (1) the inherent difficulty in studying enzymatic reactions on lipophilic substrates, and (2) the perception that the essential features of the regulation of lipolysis were already elucidated. Over twenty years ago, abundant data supported a model in which  $\beta$ -adrenergic agents lead to an increase in the intracellular concentration of the cyclic AMP that activates PKA, which in turn phosphorylates and stimulates HSL. Even the precise amino acid residue modified was established in the late 1980's as S563, although more modern analyses have suggested that S659 and S660 are the more important regulatory sites of PKA phosphorylation (Garton et al., 1988; Anthonsen et al., 1998). However, the more troublesome matter that was widely recognized but not generally studied was the conspicuous lack of correlation between the activation *in vitro* of HSL by PKA phosphorylation, which was 1.5–2-fold, and the augmentation in fatty acid release *in vivo*, generally exceeding 50-fold. Such a discrepancy often presents itself in the comparison of broken-to-intact cell regulatory processes. In this case, an important clue was provided by Hirsch and Rosen's report that cyclic AMP-stimulated lipolysis in cultured adipocytes was accompanied by a redistribution of triglyceride lipase from the soluble to a particulate fraction, affirmed a surprising eight years later by the immunologic demonstration of HSL relocation to the fat droplet in primary rat adipocytes (Hirsch and Rosen, 1984; Egan et al., 1992). From these and other studies emerged that idea that not only was activation of HSL required for maximal lipolysis, but also its translocation to substrate, the lipid droplet. However, the simplest explanation, that exposure of the enzyme's substrate-binding face would be sufficient to promote HSL redistribution, turns out not to be the case. Perhaps the clearest demonstration of this is the finding in the current work by Sztalryd et al. (2003) that HSL in fat cells lacking another critical component, the regulatory protein perilipin, is incapable of translocating to the lipid droplet after increases in cyclic AMP.

A number of years ago, evidence appeared indicating that the interface between the lipid droplet and the cytoplasm was not at all banal, but was instead characterized by an elaborate network involving filaments and tubular structures (Novikoff et al., 1980; Franke et al., 1987). Functional complexity has been more difficult to establish. However, it turns out that the most prominent phosphorylated protein in the cyclic AMP-treated adipocyte, perilipin, is localized to the periphery of the

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\*Abbreviations used in this paper: ADRP, adipocyte differentiation-related protein; HSL, hormone-sensitive lipase; PKA, protein kinase A; TAG, triacylglycerol.

lipid droplet in the basal state (Greenberg et al., 1991, 1993). Perilipin, which has been detected only surrounding intracellular neutral lipids, is expressed exclusively in adipocytes and steroidogenic cells, in which HSL serves to catalyze the cyclic AMP-driven hydrolysis of cholesterol esters to yield substrate for steroid hormone biosynthesis. Perilipin itself contains six canonical cyclic AMP phosphorylation sites, of which the amino-terminal three appear most important for function (Tansey et al., 2003). A dramatic illustration of the importance of perilipin is provided by the phenotype of mice deficient in its expression (Martinez-Botas et al., 2000; Tansey et al., 2001). These animals exhibit a reduced fat cell mass and striking resistance to obesity, as well as elevation in basal lipolysis. Nonetheless, they are severely impaired in their ability to respond to  $\beta$ -adrenergic stimulation by an appropriate elevation in circulating fatty acids. Perhaps most surprising is that mice lacking perilipin are more crippled in regard to regulation of fatty acid mobilization than those deficient in HSL (Osuga et al., 2000; Haemmerle et al., 2002). The explanation for these observations is clearly provided here using several tissue culture systems. First, they confirm that adipocytes prepared from perilipin null mice do indeed display elevated basal lipolysis in addition to resistance to  $\beta$ -adrenergic stimulation. Next, they utilize CHO cells, which normally contain neither HSL nor perilipin, to show that expression of the former is not sufficient to confer full PKA-responsive lipolysis, which instead requires the presence of both proteins. Moreover, lipolysis correlated well with translocation of HSL to the lipid droplet, serving to emphasize not only the importance of subcellular localization to TAG breakdown, but also the crucial role of perilipin phosphorylation in the redistribution of HSL. Interestingly, in the absence of perilipin, another related protein, ADRP, coats the lipid droplet, but this seems incapable of either restraining lipolysis to a minimum nor allowing HSL to migrate to the TAG in the presence of cyclic AMP. The authors understandably conclude that perilipin is endowed with at least two unique properties: the ability to prevent hydrolysis of intracellular TAG under resting conditions, but also to respond to its own phosphorylation by PKA by granting neutral lipase access to the droplet. That the latter is, at least in part, a consequence of modification of perilipin and not solely due to HSL phosphorylation is indicated by the residual cyclic AMP-stimulated lipolysis in HSL null cells, and its dependency on perilipin phosphorylation.

Nonetheless, all these important insights leave obscure the process by which perilipin governs accessibility of TAG to lipases. Although the simplest idea would be that perilipin serves as an initial docking site for HSL, its ability to accommodate other lipases suggests a more elegant explanation. Sztalryd et al. suggest as an alternative mechanism that phosphorylated perilipin somehow modifies the TAG such that it is more effectively presented as a substrate. The idea that protein-phospholipid interactions can alter the shape of the plasma membrane has long been considered plausible, though how similar alterations could be accomplished for neutral lipid remain recondite (Huttner and Schmidt, 2000). One possibility is that phosphorylated perilipin merely disperses TAG allowing easier contact by lipase, although even how this could occur is difficult to envision. Ultimately, it seems that resolving the mechanism of peri-

lipin action will be hampered by the same obstacle that has slowed the work on lipolysis: the difficulty of obtaining a clear picture of the lipid substrate. Nonetheless, the work of Sztalryd et al. bring us closer to understanding how fatty acid mobilization is regulated in the fat cell.

Submitted: 2 June 2003

Accepted: 3 June 2003

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