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# β-ADRENERGIC RECEPTORS AND REGULATION OF ENERGY EXPENDITURE: A Family Affair

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■ **Abstract** The family of adrenergic receptors (ARs) expressed in adipocytes includes three sibling βARs and two αAR cousins. Together they profoundly influence the mobilization of stored fatty acids, secretion of fat-cell derived hormones, and the specialized process of nonshivering thermogenesis in brown adipose tissue. The two types of fat cells that compose adipose tissue, brown and white, are structurally and functionally distinct. Studies on the mechanisms by which individual βAR regulates these cell-specific functions have recently uncovered new signal transduction cascades involved in processes traditionally ascribed to adenylyl cyclase/cAMP/protein kinase A system. They illustrate how βAR signaling can orchestrate a coordinated set of intracellular responses for fine control of metabolic balance.

## INTRODUCTION

Research over the past decade has provided an unprecedented expansion of our knowledge about the physiology and molecular biology of the "adipose organ." Among the newly recognized functions, adipose tissue is now appreciated to be a bona fide endocrine organ capable of secreting a plethora of biologically active substances with local and/or systemic actions (1–7). Prehaps the first and most important event in this new era of adipocyte biology was the positional cloning of the *ob* locus encoding a protein now called leptin (8), the long-sought lipostatic factor postulated 50 years ago by Kennedy (9) and supported biochemically by the elegant parabiosis experiments of Coleman (10). Expressed in adipose tissue, leptin is a genuine adiposity signal, whose pleiotropic effects include decreasing appetite and increasing energy expenditure through neuroendocrine action in the central nervous system (reviewed in References 11, 12). Another exciting new fat cell–derived factor is the adipocyte complement-related protein of 30 kDa (Acrp30)/adipoQ/adiponectin (13–15), which appears, provisionally, to possess insulin sensitizing activity. With these few examples there is a growing list of

products secreted by adipose tissue, which includes many other peptide hormone and proinflammatory cytokines (16–20), all of which are potential regulators of fuel homeostasis and suspected mediators of insulin resistance. With the clinical association between obesity and insulin resistance (21–23) and the first "prescription" for reversing early stage glucose intolerance being weight reduction (24), there has been great excitement over the discovery of fat–cell–secreted molecules that may contribute, together with free fatty acids themselves, to insulin resistance. In light of these discoveries, we must also not overshadow similarly exciting findings on the more traditional, basic role of the tissue, which is to be a temporary storage site for nonesterified fatty acids (NEFAs) that are readily accessible when required. In this review, we discuss adipose tissue heterogeneity from the macroscopic to the molecular levels and the central role of the sympathetic nervous system in lipid metabolism, and, in doing so, revisit the adrenergic control of fat cell lipolysis and thermogenesis, including some new developments in our understanding of these processes.

## STRUCTURE AND FUNCTIONAL FEATURES OF WHITE AND BROWN ADIPOSE TISSUE

### Morphological Aspects

The mammalian adipose organ is composed of subcutaneous and visceral fat depots, themselves composed of two tissue types that have critical and interrelated roles in energy balance (25–27). The main characteristics of these adipose tissue types are briefly listed in Table 1 and extensively described in the following paragraphs. White adipose tissue (WAT) is populated mainly by white adipocytes and yet can contain a variable amount of brown adipocytes (28–33). Conversely, brown adipose tissue (BAT) is composed almost exclusively of brown adipocytes. Cells in certain fat depots appear to be able to "change" between the white and brown adipocyte phenotype in an age- or environment-dependent manner (27, 29, 30, 32–34). Now under debate in the field is the possibility of transdifferentiation between the WAT and BAT phenotype. Although seemingly heretical, if true, it adds another level of plasticity to the adipose organ (35). Both adipose tissue types are able to store NEFAs as triacylglycerol (TG), but whereas WAT TG hydrolysis satisfies the energy needs of the whole organism, fatty acids released from BAT are used within the tissue to promote nonshivering thermogenesis (36–39).

WAT is the predominant type of adipose tissue in adult mammals; its amount usually increases with age, and in obese individuals it can account for more than half of total body weight. In healthy adult humans, it accounts for 15%–20% of body weight in men and 20%–25% in women. The principal cell type of WAT contains a single (unilocular) and large (20–200 $\mu$m) lipid droplet, resulting in the near disappearance of the cytoplasm and compression of the nucleus underneath the plasma membrane (40). These cells are grouped into small

**TABLE 1** Some basic characteristics of WAT and BAT

| | **WAT** | **BAT** |
|---|---|---|
| Vascular system[a] | + | ++(TN), +++(CE) |
| SNS innervation | ++ | +++ |
| White adipocytes | +++ | ++ |
| Brown adipocytes | + | +++(rodents), +/−(human)[b] |
| Mitochondrion | + | ++(TN), +++(CE) |
| Lipid droplets | Unilocular | Multilocular |
| BARs subtypes | +++($\beta$3), +($\beta$1), +/−($\beta$2) in rodents<br>+++($\beta$2), ++($\beta$1), +/−($\beta$3) in human[c] | +++($\beta$3), +($\beta$1), +/−($\beta$2) in rodents<br>+++($\beta$2), ++($\beta$1), +($\beta$3) in human[c] |
| Leptin | +++ | +/− |
| Type II deiodinase activity | +/− | +(TN), +++(CE) |
| PGC-1$\alpha$ | +/− | +(TN), +++(CE) |
| UCP1 | +/− | +(TN), +++(CE) |
| UCP2 | + | + |
| UCP3 | +/− | + |

TN, thermal neutrality; CE, cold exposure.

[a]References are listed in the text.

[b]BAT in adult human is restricted to the mixed type perirenal adipose tissue.

[c]Because no good antibody or human specific ligands existed when assessed, this should be revisited.

lobules surrounded by connective tissue septae. WAT is considered to be less well vascularized than BAT, but, nevertheless, it displays an extensive capillary bed reaching each septae and therefore most adipocytes (41, 42). Anatomical and histologic observations show direct sympathetic innervations of white adipocytes originating from the central nervous system (43, 44). Most of these catecholaminergic nerves are in proximity to the vasculature (45, 46). Visceral fat depots appear to be more richly innervated in contrast to the subcutaneous fat depot (47).

BAT is a thermogenic organ populated by small (20–40 $\mu$m) adipocytes characterized by numerous (multilocular) lipid droplets. BAT is present in essentially all mammals at birth and is responsible for diet- and cold-induced nonshivering thermogenesis (48–50). The distinctly russet tint of BAT derives from its rich vasculature, each adipocyte receiving up to five capillaries, and to its population of brown adipocytes, which are densely packed with large mitochondria (25, 42, 51). Within these mitochondria resides a unique molecule that has the ability to allow a proton leak and, therefore, uncouple oxidative respiration from ATP production (52, 53). Also previously called thermogenin (37, 54), the gene for this protein

was cloned almost 20 years ago and is now termed uncoupling protein or UCP1 (55). In most mammals, BAT is most abundant during the perinatal period. But in contrast to rodents, which retain a distinct BAT depot in the interscapular region, adult humans do not have homogenous BAT depots. Nevertheless, one can observe variable quantities of brown adipocytes dispersed through several of the typical WAT depots in humans (30, 31). BAT is very richly innervated by the sympathetic nervous system (SNS), much more than WAT, as both the vascular system and the adipocytes are abundantly innervated (56–58).

## Molecular Aspects of Adipogenesis and $\beta$-Adrenergic Receptor Expression

Extensive studies of white preadipocyte cell lines, such as the 3T3-F442A and 3T3-L1, have concluded that, at least for white adipocytes, two families of transcription factors are largely responsible for the commitment to and maintenance of adipocyte differentiation: the CCAAT/enhancer binding proteins (C/EBPs) (59–65) and peroxisome proliferator-activated receptor $\gamma$ (PPAR$\gamma$) (66–68). This topic has been extensively reviewed by others (69–72).

Regarding the $\beta$ARs in adipocytes, the $\beta_1$AR and $\beta_2$AR are present at low levels in the preadipocyte stage (73–76). The $\beta_3$AR is expressed only upon full differentiation (77, 78); this is due to an absolute requirement for the transcription factor C/EBP$\alpha$ (78). Although there is widespread need by most adipocyte genes for PPAR$\gamma$, curiously, the 5.3-kb fragment of the mouse $\beta_3$AR promoter can appropriately target expression of the receptor to WAT and BAT in vivo (T.L. Martin & S. Collins, unpublished), but binding sites for PPAR$\gamma$ have not been found.

Although the cascade of transcriptional events driving adipogenesis in white fat has been extensively studied, this is not the case for brown fat. The adipogenic white adipocyte cell lines have existed for over 25 years (79), but equivalent cell culture models of brown fat have come into existence only in the past few years (80–82), and yet most do not mimic endogenous BAT quite as well as white adipocyte models do. For this reason, studies on brown fat have lagged. Moreover, recent results suggest that the events involved in brown adipocyte differentiation will not necessarily mirror those in WAT. For example, mice with a targeted disruption of the C/EBP$\alpha$ gene lack detectable WAT, but they nevertheless appear to have differentiated BAT as defined by morphological criteria and expression of UCP1 (83). Although the factors distinguishing WAT from BAT are not known, one molecule that is likely to be a key player is a nuclear coactivator isolated from BAT with a role in regulating mitochondriogenesis and oxidative metabolism called PPAR$\gamma$ coactivator-1 (PGC-1$\alpha$) (84). PGC-1$\alpha$ is readily detected in brown fat, but only very weakly expressed in WAT. Consistent with a role for this coactivator in the phenotypic distinctions between these cell types, ectopic expression of PGC-1$\alpha$ in cultured white adipocytes induces genes that are associated with the BAT phenotype, including UCP1 and components of the electron transport chain. Its expression in vivo is also cold-inducible in BAT and skeletal muscle, and this stimulation

is mediated by increases in cAMP (84, 85). For the $\beta$ARs, there is a very similar pattern to the control of their expression, although the relative amounts of $\beta_1$AR are substantially greater, and the triplet transcripts for $\beta_3$AR show a distinctly different pattern that favors the smaller 2.1 kb form (Figure 1). The molecular basis of these different transcripts are not fully defined, but do not appear to alter the coding sequence of the protein and are more likely associated with differential splicing with untranslated portions of the transcript (86). There is some evidence for alternative transcription start sites (87, 88), but this has not been confirmed.

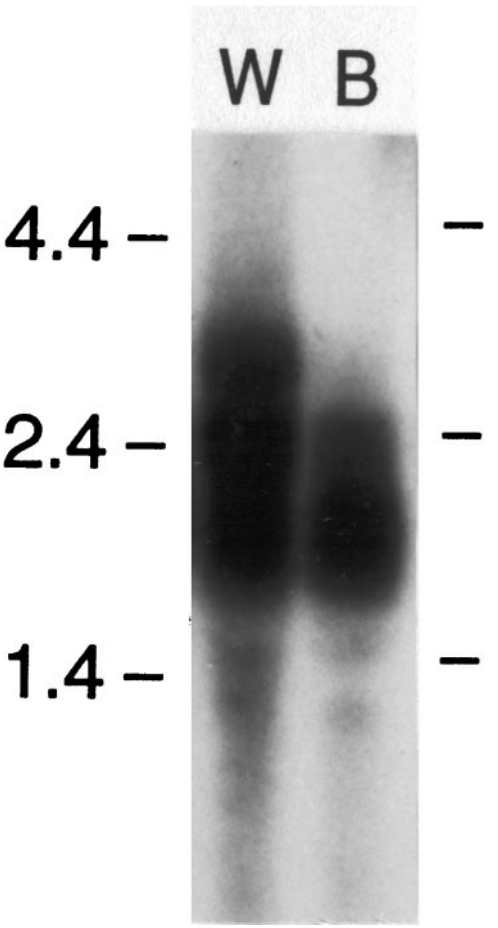



**Figure 1** Transcripts for $\beta_3$AR in WAT and BAT. Northern blot showing the three distinct species of mRNAs for the $\beta_3$AR (86, 183). The relative abundance of these transcripts differs between WAT (*W*) and BAT (*B*). Size markers adjacent to the image are in kilobases.

## ROLE OF THE SYMPATHETIC NERVOUS SYSTEM AND $\beta$-ADRENERGIC RECEPTORS IN LIPOLYSIS

When nutrients are plentiful, adipocytes synthesize and also take up NEFAs, which are then esterified and routed inside lipid droplets in the form of triacylglycerol. The amount stored reflects the cumulative sum over time of the differences between energy intake (food consumption) and energy expenditure (physical activity as well as obligatory and adaptative thermogenesis, discussed below) (89). The net efflux of NEFAs from adipose tissue alternates between being maximal after an overnight fast or during a bout of exercise to being minimal or nonexistent 60–120 min after a meal (90, 91). The raison d'être of this fluctuation is first to meet minute-to-minute metabolic demands. It can be postulated that it also serves to protect nonadipose tissue against "lipotoxicity": inappropriate accumulation of NEFAs with adverse effects on fuel metabolism and, ultimately, on health (92).

In times of net caloric deprivation, whether it occurs in response to extended food scarcity or fasting, sustained intense physical activity, or even during the latter hours of sleep (overnight fasting), the drop in blood glucose triggers the SNS to release the catecholamines epinephrine and norepinephrine (93). It is well established that both WAT and BAT are innervated by the SNS (45, 94, 95). However, because sympathetic innervations are more profuse in BAT than in WAT, neural-derived norepinephrine is presumed to play a greater role in the former, whereas catecholamines derived from the circulation play a relatively greater role in WAT. Nevertheless, there can be significant levels of norepinephrine in the immediate vicinity of the nerve terminals. Another situation where norepinephrine turnover is acutely regulated in WAT is during cold exposure (96); this is discussed together with the regulation of BAT nonshivering thermogenesis.

The role of the SNS in the control of lipolysis has been investigated in many ways through the past century and thoroughly reviewed recently (44). Briefly, these studies showed that denervation of white fat depots lead to tissue hypertrophy (97, 98) and, reciprocally, that electrical stimulations of these WAT nerves led to fatty acid release (99, 100). The responses elicited by these electrical stimulations were blocked by manipulations that prevented norepinephrine release or norepinephrine binding to $\beta$ARs and were potentiated by $\alpha$-adrenergic blockers and inhibitors of phosphodiesterases (99, 101, 102). Thus, upon SNS stimulation, norepinephrine is released, binds to $\beta$ARs to activate adenylyl cyclase, and ultimately stimulates lipolysis.

The $\alpha$ARs and the $\beta$ARs are the recipients of these catecholamine signals. They are members of the large family of G-protein coupled receptors that are integral membrane proteins of the plasma membrane. There are three subtypes of $\beta$ARs ($\beta_1$AR, $\beta_2$AR, and $\beta_3$AR) (103, 103a, 104) all of which are expressed in white and brown adipocytes (86, 105–107). However, the relative proportions of these subtypes vary between species, fat depots, and metabolic status (108). For example, rodent species possess abundant levels of the $\beta_3$AR and lesser amounts of the two

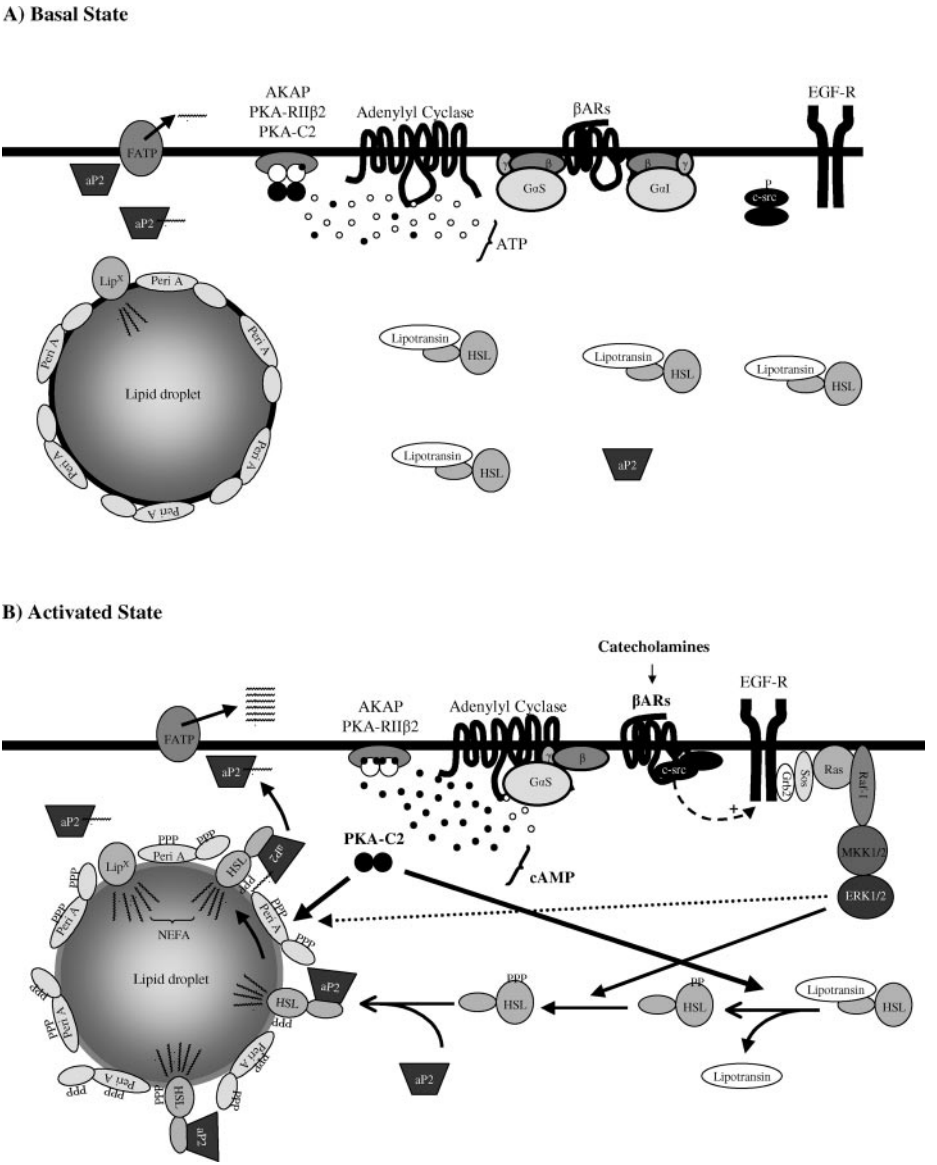
other subtypes, whereas the reverse is generally the case in humans, although there is clearly a need to examine intraabdominal depots in normal humans more carefully.

The control of lipolysis by the $\beta$ARs is principally initiated by the sequential activation of adenylyl cyclase and cAMP-dependent protein kinase (PKA), ultimately culminating in the phosphorylation of hormone-sensitive lipase (HSL) and perilipin A (109–113). In addition to the $\beta$-adrenergic stimulation of lipolysis, catecholamines can also be antilipolytic through their interaction with the $\alpha_2$ARs and its resulting inhibition of cAMP production. The balance between the relative amounts of the $\beta$AR and $\alpha_2$AR can thus determine the relative efficacy of catecholamines for triglyceride hydrolysis. In that respect, there is some evidence from experimental studies in animals and humans that a shift to a higher $\alpha_2/\beta$ ratio can contribute to obesity and net lipid storage (114).

Rat and human HSL are 82.8 and 84 kDa proteins, respectively (115, 116). By SDS gel electrophoresis, they migrate at an apparent molecular weight of 84 and 88 kDa, respectively, but under nondenaturing conditions, higher oligomer forms have been observed (117). The significance of this oligomerization, and whether it is artifactual, is not clear. By limited proteolysis and mutagenesis of HSL, it has been suggested that the carboxy-terminal domain contains a catalytic triad composed of Ser-423, Asp-703, and His-733, as well as the regulatory Ser-563, Ser-600, Ser-659, and Ser-660 (118–121). Interestingly, Ser-600 is not a substrate for PKA but rather for the extracellular signal-regulated kinases 1 and 2 (ERK) (121). PKA phosphorylation of Ser-563 was once thought to be solely responsible for enhanced HSL catalytic activity, but careful mutagenesis experiments convincingly showed that Ser-659 and Ser-660 are the major sites of PKA regulation (120). Nevertheless, because studies in vitro on phosphorylation-dependent catalytic activity of HSL are modest (two- to threefold), they cannot explain the usually more dramatic effect of PKA on lipolysis in vivo (122, 123). More recently, it has become apparent that a significant fraction of the PKA-dependent activation of lipolysis relies on the translocation of the lipase to the surface of the lipid droplet (113, 124–126). Ser-659 and Ser-660 of HSL have been described as obligatory for this process (127), as well as the phosphorylation of Ser-81, Ser-222, and Ser-276 on perilipin (128). The exact mechanism of this regulation is not clear yet, but the tight association of perilipin with the triglyceride droplet (129–131) appears to be a tonic inhibitor of basal lipolysis (132, 133). Upon phosphorylation, perilipin would seem to "loosen it's grip" on the lipid droplet (113, 134, 135) and, combined with a direct interaction with HSL, favor access of the lipase to its substrate. Nevertheless, if there is a surface monolayer of phospholipids as proposed (130), an additional level of regulation may be necessary in order for HSL to access the triacylglycerol core. With evidence that ERK can phosphorylate Ser–600 of HSL, we might speculate that it could favor protein-protein interactions with as yet unknown partners required for full lipolytic activity. Figure 2 is a synthesis of the regulation of lipolysis by the $\beta$ARs.

## DIVERSITY OF $\beta$-ADRENERGIC SIGNALING MECHANISMS OF ADIPOCYTES

The activation of PKA by $\beta$ARs is well established. Here, we describe the activation of various MAP kinase cascades in addition to this well-established cAMP/PKA pathway. These include ERK1/2 MAPK and p38 MAPK. They are independent of



each other in white and brown adipocytes (136) and activated in response to catecholamines by different mechanisms. The activation of the ERK1/2 MAP kinases by β-adrenergic agonists in white and brown adipocytes is controversial; we have shown that it occurs as a result of receptor coupling to the heterotrimeric G protein Gi (137) and does not involve PKA (136), which have both been corroborated by some investigators (138) but not by others (139). The nature of the discrepancy can be multiple, but the most obvious is the use by our colleagues of BRL-37344 instead of CL316243. On the other hand, p38 MAPK activation is downstream of β-agonist increases in cAMP levels and PKA activity (136, 140). The ERK pathway appears to account for 15%–25% of total lipolysis (121) (Figure 3**)**. Our pharmacologic analyses suggest that at low catecholamine concentrations, essentially all lipolysis is activated by PKA, whereas the ERK1/2 pathway may be most significant at higher concentrations of norepinephrine.

Although we have yet to establish the functional consequences of p38 MAPK activation in white adipocytes, there is a very clear indication that in brown adipocytes, the classic cAMP-dependent stimulation of the UCP1 gene requires this pathway (136). All of these new findings require additional studies to establish the metabolic ramifications of these simultaneous signaling cascades emanating from βARs, and there must be follow-up studies using primary adipose tissue samples from humans in order to assess the importance of these pathways in humans. Other evidence indicates that the adipocyte-derived hormone leptin is also regulated by the catecholamines. In animal models, as well as in humans, the secretion of leptin is decreased by β-agonists (141–143) but the mechanism(s) responsible for this suppression by βARs, including how leptin secretion is regulated in general, is not yet understood.

**Figure 2** Mechanisms of WAT lipolysis stimulation by βARs. In the basal state (*A*), nonphosphorylated HSL is in the cytosol, probably bound to some cytosolic acceptors such as lipotransin, and nonphosphorylated perilipin is tightly bound to the lipid droplet. HSLs do not have free access to the droplet, so most of the basal lipolysis is attributed to a nonidentified additional lipase. (*B*) When catecholamines interact with the βARs, they can alternatively couple to both Gs and Gi (137, 138, 168). The former lead to the sequential stimulation of adenylyl cyclase and PKA, which is bound via its regulatory subunits to juxtamembranous anchor proteins called AKAPs. The catalytic subunits of PKA can then access both HSL, which is phosphorylated at two serine residues in the regulatory region (Ser-659 and Ser660), and perilipin, which is phosphorylated at six serine residues, three in the regulatory region (Ser-81, Ser-222, and Ser-276) and three in the carboxy-terminal portion (Ser-433, Ser-494, and Ser-517). On the other hand, Gi activation leads to EGF receptor transactivation and activation of the ERK pathway. The ERK pathway, in turn, can also lead to the phosphorylation of HSL (Ser-600) and probably perilipin.

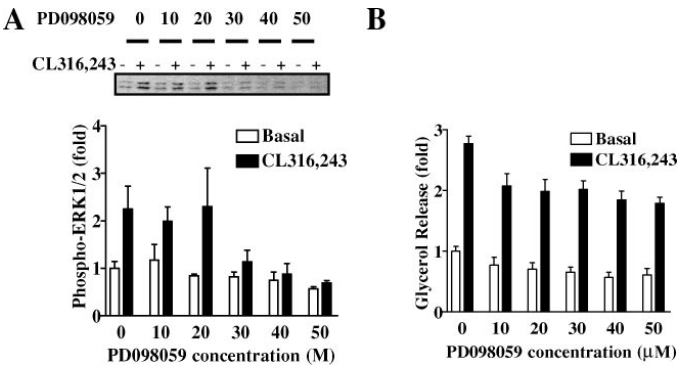



**Figure 3** Contribution of ERK to $\beta_3$AR-agonist stimulated lipolysis in white adipocytes. Differentiated 3T3-L1 cells were pretreated with increasing concentrations of the MEK1/2 inhibitor PD098059 for 1 h, followed by the addition of CL316243 (100 nM). (*A*) ERK activation was measured by antiphospho ERK1/2 immunoreactivity and (*B*) lipolysis was measured by assessing the glycerol content of the media. Results are from three independent experiments.

## THERMOGENESIS AND THE RELATIVE ROLES OF WHITE AND BROWN ADIPOSE TISSUE IN BODY COMPOSITION AND ENERGY HOMEOSTASIS

In mammals, including humans, total energy expenditure represents the sum of the obligatory ATP utilized to sustain life, to generate muscular activity, and to respond to the surrounding environment. The main component, which is obligatory energy expenditure or obligatory thermogenesis, mostly refers to the basal metabolic rate that has a significant thyroid hormone-regulated component (reviewed in Reference 144). This basic cost is attributed to nucleic acid synthesis and substrate cycling (30%–35%), protein turnover (20%–25%), sodium and potassium pumping (20%–25%), gluconeogenesis (7%), calcium pumping (5%), the actomyosin ATPase (5%), and ureagenesis (2.5%) (145). Also included in the obligatory component of thermogenesis is the thermic effect of food, the effort required to digest and absorb nutrients. Measured thermic effects of nutrients are 0%–3% for fat, 5%–10% for carbohydrates, and 20%–30% for proteins (146). Energy expenditure associated with physical exertion occurs mainly within the skeletal muscle, with a small but nonnegligible role of the liver. Finally, facultative or "adaptive" thermogenesis is that which can be modulated by the environment and occurs in both muscle and BAT in the form of shivering and nonshivering thermogenesis, respectively.

In response to cold exposure or overfeeding, mammals exhibit a complex response marked by increases in oxygen consumption, food intake, and heat generation through nonshivering thermogenesis in BAT. An immense body of work

has shown that BAT is uniquely capable of responding to various environmental stimuli to generate heat from stored metabolic energy (55, 147–157). The major mechanisms for all these responses involve SNS activation, especially during cold exposure (151, 158, 159).

In response to sympathetic nervous system activation, BAT undergoes an orchestrated hyperplastic and hypertrophic expansion, increased blood flow, and recruitment of lipid and carbohydrate fuels for oxidative metabolism (152, 160). A unique and critical element of this thermogenic mechanism for dissipation of the proton gradient in brown fat mitochondria was recognized to be due to a brown fat–specific mitochondrial uncoupling protein (UCP). This uncoupling activity in brown fat mitochondria is acutely under the control of the free fatty acids (FFAs) that are released as a result of catecholamine-stimulated lipolysis (161–163). At the cellular level, this "recruitment" of brown fat for thermogenesis occurs in response to noradrenergic stimulation and includes brown adipocyte proliferation, increased expression and activity of the brown adipocyte–specific UCP1, and mitochondrial biogenesis (164). All of these responses are mediated by the $\beta$ARs, with lesser supporting contributions from the $\alpha_1$AR. The proliferative expansion of BAT is mediated by the $\beta_1$AR (152, 165) and depends upon elevations of intracellular cAMP levels (160). Because the cell population from which these new brown adipocytes derive is a precursor, they express $\beta_1$AR and $\beta_2$AR, but not $\beta_3$AR, the latter being expressed only in differentiated adipocytes. It is not known conclusively whether there is a unique signaling cascade emanating from the $\beta_1$AR or whether it is simply the result of the greater sensitivity of $\beta_1$AR versus $\beta_2$AR for norepinephrine released from the nerve terminals innervating BAT. There is also evidence that, at least in fetal rodent brown adipocytes, MAP kinase cascades might be involved, as there is evidence for growth promotion and protection from apoptosis in that model (166). The transcriptional activation of the UCP1 gene appears to depend upon the combined stimulation of cAMP/PKA irrespective of the $\beta$AR subtype, the p38$\alpha$ MAP kinase, and the recruitment and p38-dependent activation of PGC-1$\alpha$ (W. Cao, K.W. Daniel, J. Robidoux, P. Puigserver, A.V. Medvedev, X. Bai, L.M. Floering, B.M. Spiegelman, S. Collins manuscript submitted). Based upon what is also known about PGC-1$\alpha$ orchestrating the program of mitochondriogenesis (85), it is also very likely that the increased expression and activation of PGC-1$\alpha$, which occurs as a consequence of $\beta$AR-stimulation and p38 MAPK activation (84; W. Cao, K.W. Daniel, J. Robidoux, P. Puigserver, A.V. Medvedev, X. Bai, L.M. Floering, B.M. Spiegelman, S. Collins manuscript submitted), will explain this aspect of SNS-stimulated thermogenesis. Figure 4 relates our understanding of the regulation of UCP1 by the SNS.

A role for brown fat thermogenesis in the regulation of body composition has been discussed since the first reports describing how weight gain in overfed animals was insufficient to account for the net calories consumed. Brown fat was found to be the organ in which this apparent energy dissipation occurred, and the process was dubbed diet-induced thermogenesis (see, e.g., Reference 149). The discovery

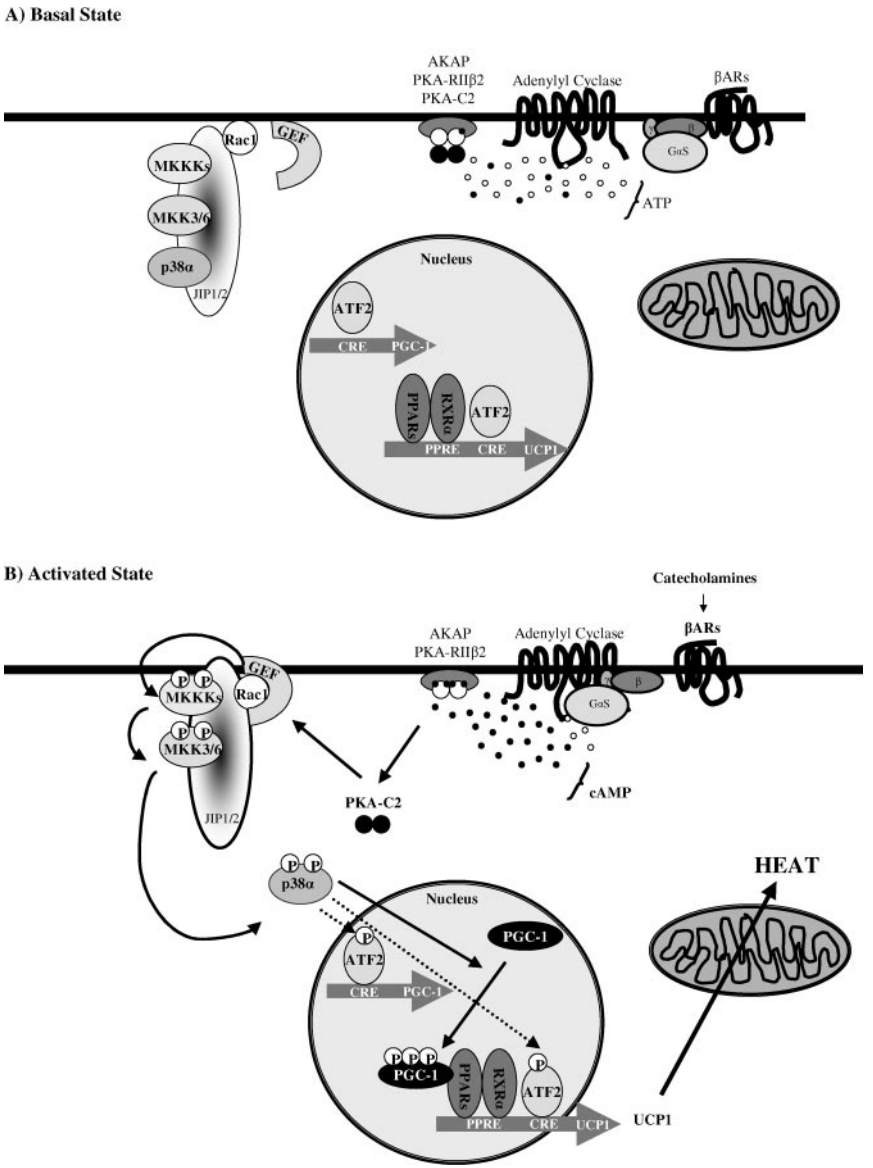



**Figure 4** Mechanisms of BAT thermogenesis stimulation by $\beta$ARs. Under the basal state, the PKA and p38 MAPK pathways are quiescent. When catecholamines interact with the $\beta$ARs, they lead to the sequential stimulation of adenylyl cyclase and PKA, which in turn activates a specific protein kinase cascade, culminating in the activation of p38$\alpha$, and thus activation of a subset of transcription factor including ATF2. A second phase response ensues in which newly transcribed PGC-1 transactivates members of the peroxisome proliferator activated receptor (PPAR) family and therefore UCP1 expression.

of thermogenic $\beta$-adrenergic agonists in the mid-1980s that seemed to target BAT (discussed further below and in Reference 168) further stoked enthusiasm that a tissue classically responsible for the adaptation to prolonged cold could also play a role in modulating body composition: the relative ratio of lean to fat mass. When UCP1 was identified as the molecule within BAT mitochondria responsible for uncoupling oxidative metabolism from ATP production, experiments were devised to see if simply overexpressing this molecule in white fat could achieve a similar end. The results were both fantastic and intriguing. Kozak & Kopecky generated mice that expressed UCP1 from the adipocyte aP2 promoter (169). These animals were very lean, were resistant to diet-induced obesity, and could maintain normal glucose tolerance in the face of the high-fat dietary challenge (170, 171). What they didn't necessarily expect was that the expression of the endogenous UCP1 gene in brown adipocytes would be severely blunted (169). At this same time, a different experiment in which BAT was ablated by a transgenic manipulation also resulted in animals that were obese, hyperphagic, and insulin resistant (172). Moreover, in certain founder mice, the brown adipocytes were able to escape the effects of the diphtheria toxin transgene. These animals then slimmed back down and returned to normal, once again supporting the notion that thermogenesis in BAT can control body composition. Others explored the hypothesis that if one enhanced the flow of metabolites through futile intermediary pathways, this would also serve as another means of thermogenesis. Indeed, Kozak & colleagues (173) showed that transgenic overexpression of glycerol 3-phosphate dehydrogenase resulted in abnormal development of WAT and BAT such that the BAT depot was enhanced relative to WAT, rendering the animals increasingly lean with age. Moreover, this response was independent of UCP1, whose expression was greatly downregulated. In view of these and other studies supporting a role for thermogenesis, BAT in particular, in body weight regulation, it was quite surprising that mice with a targeted disruption of the UCP1 gene were in fact not obese, but were quite sensitive to the cold (174). At this time, other members of the UCP family had been discovered (reviewed in Reference 175), with suggestions that they may function analogously to UCP1 to dissipate excess caloric intake in mice prone to diet-induced obesity (176). It now appears, after several years of intensive research on these proteins, that their function in the mitochondria is not akin to UCP1 (177). Instead, UCP2 and UCP3 play a role, still undefined, in lipid oxidation in the mitochondria. The plot has thickened in the continuing saga of BAT and UCP1, thermogenesis, and body composition with the report that UCP1-deficient mice are in fact less prone to diet-induced obesity (178) than their wild-type counterparts, an observation we have also made in support of these findings (L.M. Floering & S. Collins, unpublished). From these new studies, there is now discussion in the field that in rodents, the interscapular BAT depot and its unique molecule UCP1 comprise an efficiently designed stove for generating heat from metabolic fuel in times of environmental challenges. The more intriguing question thus arises, What is the mechanism of the diet-induced thermogenesis in the UCP1-deficient mouse? The answers will no doubt be interesting.

## THE REGULATION OF $\beta$ARs IN ADIPOSE TISSUE AND ROLE IN OBESITY

We have gained much information about obesity and its metabolic consequences from the monogenic models in rodents, without which much of the work today on adipocyte biology, obesity, and insulin resistance could not have been accomplished. With regard to fat metabolism, it was known for many years that the genetically "obese" C57BL/6J *ob/ob* mouse (now known as the leptin-deficient C57BL/6J$^{Lep\text{-}ob/Lep\text{-}ob}$) exhibited a marked inability to effectively mobilize triglycerides from WAT and was unable to recruit BAT for thermogenesis in response to cold temperature (reviewed in Reference 179). Because at the time it was generally accepted that only a single $\beta$AR subtype existed in adipose tissue (180, 181), efforts to understand the dysregulated $\beta$AR responses in adipocytes met a dead-end. As more selective sympathomimetic agents were developed that could distinguish between $\beta_1$- and $\beta_2$ARs, this view began to change. With the discovery of the $\beta_3$AR in 1989 (103, 103a, 104), we reevaluated the expression and function of the adipocyte $\beta$ARs from lean versus leptin-deficient *ob/ob* mice and found a dramatic decrease in both $\beta_1$- and $\beta_3$ARs mRNA levels. Through a series of detailed pharmacologic analyses, these changes in $\beta$AR subtype expression were shown to be responsible for the inability to mobilize stored fat in response to $\beta$-agonists (86).

Other models of congenital obesity, such as *db/db*, *tubby*, *fat*, and the Zucker fatty rat, show similar decreases in $\beta_3$AR and $\beta_1$AR expression, the extent of which tends to mirror the severity of obesity (182, 183). However, the vast majority of human obesity is not due to single gene mutations, but is most often a result of dietary excess and the resulting metabolic complications. As a research tool to more adequately reflect this fact, nonmutant C57/BL6J (B6) mice raised on a high-fat diet (184) show similar defects in $\beta$AR function and expression in adipocytes (185). Thus, in essentially all models of obesity, there is a significant diminution in the expression and function of the $\beta$ARs.

The notion that one could develop therapeutic agents to stimulate thermogenesis as antiobesity and antidiabetic treatments arose out the discovery of $\beta$-adrenerigc compounds that could increase oxygen consumption and cause selective loss of white adipose stores in the extremely obese mutant *ob/ob* mouse (186). Much of the history of these discoveries and the unique effects of $\beta_3$AR-selective agonists as thermogenic agents have been previously reviewed (168). When treated in the laboratory with $\beta_3$AR-selective agonists, a variety of mammals exhibit a vigorous thermogenic response akin to cold exposure, supporting the notion that the $\beta_3$AR plays a significant role in this thermogenic response (185, 187–192). However, perhaps the most puzzling but immensely intriguing feature observed is the de novo appearance of brown adipocytes within typical white adipose depots, suggesting a close interplay between these two adipocyte species. The source of these brown adipocytes is unknown. They may arise from proliferation, but no evidence in support of this can be found (193). There is currently discussion in the field that small pockets of dedifferentiated brown adipocytes from the neonatal

period may be present in white adipose depots, expressing very low amounts of $\beta_3$AR, but which might be triggered to redifferentiate (26). However, as discussed above, although this response to catecholamines clearly has a predominant cAMP component, other evidence indicates that MAP kinase pathways are activated by these $\beta$ARs in adipocytes (136, 137, 194), and the targets of these pathways may also contribute to the sympathetically driven brown adipocyte growth and differentiation.

Related to the improvements in body composition that were observed in animals treated with $\beta_3$AR agonists came further support for a direct role of adipocytes in regulating systemic glucose homeostasis (185, 195, 196). Because $\beta_3$AR are expressed almost exclusively in fat, effects of these agents are expected to be initiated by alterations in fat metabolism. Treatment with Cl316243 results in enhanced sensitivity of both whole-body glucose uptake and suppression of hepatic glucose production (195). These effects are accompanied by increased glucose uptake in adipose tissue (WAT and BAT) with no effect in multiple muscle groups studied (197). Thus, increasing glucose uptake selectively in fat with $\beta_3$AR agonists may improve whole-body glucose uptake, with the effects in fat indirectly resulting in increased insulin sensitivity in liver. In addition, $\beta_3$AR agonists may be efficacious by changing the release of some adipocyte product that influences systemic insulin sensitivity.

In response to these metabolic improvements, pharmaceutical companies have generated a series of human $\beta_3$AR agonists and antagonists (198–201). Treatment of rhesus monkeys with L-755507, a potent and selective partial agonist for both the human and rhesus monkey $\beta_3$AR, resulted in increased lipolysis, elevated metabolic rate, and increased expression of UCP1 in brown adipose tissue (190). The antagonists were shown to inhibit agonist-induced lipolysis in cells expressing cloned human $\beta_3$ARs and in isolated nonhuman primate adipocytes. The use of cloned human $\beta_3$ARs as screening tools have enabled the discovery of more selective human $\beta_3$AR agonists (199, 201, 202) and should lead to further improvements in potency and pharmacokinetics in the future.

## SUMMARY

Work on the molecular aspects of catecholamine-stimulated adipose tissue metabolism and thermogenesis is built upon the august foundations of earlier biochemical and physiological investigators. We have tried to infuse our review of new developments in the field with a historical outlook that allows one to understand the current molecular breakthroughs in fat cell biology with an eye toward the unanswered questions that we must attend to in the future. These include the molecular decisions during mesenchymal development that distinguish white from brown adipocytes, and how signaling pathways triggered by neurotransmitters and peptide hormones or growth factors are able to integrate their signals and their common signaling molecules to manage the relatively irregular patterns of fuel acquisition

and expenditure. Current and future discoveries at the molecular level in adipocyte biology will ultimately be proven or denied in the world of living animals and humans. We hope that our growing appreciation of the delicate fuel homeostasis of the adipose organ will lead to promising avenues of therapeutic intervention in order to minimize adipose mass enlargement and at the same time prevent overflow of NEFAs to nonadipose tissues.

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