Metabolic and cellular plasticity in white adipose tissue I: effects of \( \beta_3 \)-adrenergic receptor activation

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\( \beta_3 \)-adrenergic receptors (Adb3) are G protein-coupled receptors that are abundantly expressed in rodent adipose tissues. When given chronically, selective Adb3 agonists exert potent anti-diabetes and anti-obesity effects in rodent models (2, 7, 12, 30). Although it is not clear how Adb3 agonists produce anti-diabetes effects, a prominent feature of chronic Adb3 activation is remodeling of white adipose tissue (WAT; see Refs. 10, 11, 13, and 17). Central features of this remodeling include dramatic elevation of metabolic rate, mitochondrial biogenesis, and induction of genes involved in oxidation of fatty acids (13, 17). Significantly, chronic Adb3 activation elevates whole animal and WAT metabolism in mice lacking uncoupling protein (Ucp1), the molecular effector of brown fat thermogenesis, indicating this effect can occur independently of brown adipocyte recruitment in white fat depots (13).

The potential ability to expand the catabolic activity of WAT has important implications for anti-diabetes and anti-obesity therapeutics because adult humans are thought to possess little functional brown adipose tissue (BAT). As a step toward understanding the mechanisms involved, we performed a detailed time course analysis of WAT cellular plasticity using histological, metabolic, and global expression profiling analyses. The results indicate that Adb3 activation triggers a transient inflammatory response that is followed by mitogenesis among stromal cells and multilocular adipocytes. Chronic Adb3 activation expanded the oxidative capacity of adipocytes within WAT by inducing mitochondrial biogenesis and upregulating genes involved in fatty acid oxidation and mitochondrial electron transport activity. Analysis of gene expression patterns indicated that inflammation and adipocyte-specific gene expression were reciprocally related over time and suggested that upregulation of catabolic activity is critical to suppressing inflammation and restoring phenotypic expression in adipocytes during continuous Adb3 stimulation.

METHODS

Animals. C57Bl/6 mice and 129S1/SvImJ mice were obtained from Jackson Laboratories. C57Bl/6 mice lacking Ucp1 (Ucp1 knockout) were obtained from Dr. L. Kozak (Pennington Institute). All mice were bred at Wayne State University and used when 3–5 mo old. Animals were matched for age, and only male mice were used. Mice were maintained at 23°C in a 12:12-h light-dark cycle and had continuous access to food and water.

For histological analysis, C57Bl/6 mice were implanted with osmotic minipumps that delivered vehicle (control) or CL-316,243 (CL) at a rate of 3 nmol/h for 1, 3, or 6 days. Animals were killed, and epididymal white adipose tissue (EWAT) was removed and processed as follows.

A portion of tissue was fixed in 4% paraformaldehyde, embedded in paraffin, and processed for histological analysis as indicated for the methods of Figs. 1–6. Adipocyte cell diameters were determined by capturing brightfield images with a digital camera and measuring >100 adjacent cells in 5-µm-thick sections for individual mice using Image Pro Plus software (14). The average values for individual mice of a given treatment were then averaged for presentation.

A portion of tissue was stained with Mitotracker Red, fixed, and imaged by confocal or wide-field microscopy (13). NADH/NADPH dehydrogenase activity was detected histochemically by submerging whole EWAT pads in PBS containing 1% 2,3,5-triphenyltetrazolium chloride (TTC) for 15 min at 37°C, followed by fixation in PBS containing 4% paraformaldehyde (26, 28).

For measurement of tissue respiration, EWAT from control and CL-infused mice was placed in HEPES-buffered Krebs-Ringer solution containing 1% BSA and minced into 5- to 10-µg fragments. Minces were transferred to a closed chamber containing 700 µl Krebs-Ringer buffer for measurement of oxygen consumption with a Clark-style electrode (Qubit Systems, Kingston, ON, Canada) at 35°C under constant stirring. Oxygen consumption rates were determined by linear regression of the oxygen consumption traces (Vernier Software, Beaverton, OR).

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Bromodeoxyuridine labeling in vivo. In parallel experiments, B16 mice were infused with bromodeoxyuridine (BrdU) (20 μg/h) along with CL, as described above. EWAT, BAT, and intestine (positive control) were harvested after 3 and 7 days of infusion. In a separate experiment, control mice and mice treated with CL for 3 days were injected two times with BrdU (4 mg) at 3-h intervals, and tissues were harvested 6 h after the first injection. Fixed tissue was processed as described above. BrdU immunohistochemistry was performed on 6-μm-thick paraffin sections. Briefly, dewaxed sections were treated with 4 M HCl, rinsed, and then incubated with rat monoclonal anti-BrdU antibodies (Abcam, 1:50). Binding of primary antibodies was detected with biotinylated donkey anti-rat antibodies linked to streptavidin-alkaline phosphatase (Jackson Immunoresearch) using fast red (Biomeda) as the chromogen.

Total mitotic index was determined by counting total and BrdU-positive nuclei in at least six nonoverlapping fields at ×40 magnification and calculating an average index per mouse. Values of individual mice were then averaged for presentation. BrdU incorporation among cell types was determined by counting incorporation into adipocytes (unilocular and multilocular) and endothelial cells, which were readily identified by morphology. Stromal/adipophage cells were calculated as the residual.

Global expression profiling analysis. For analysis of mRNA, EWAT was immediately frozen and held at −80°C until processed. Global expression profiling analyses were performed in two independent experiments. In both experiments, total RNA was extracted from individual EWAT pads as detailed (13). Equal amounts of total RNA were pooled from five to nine mice, re-extracted in phenol, precipitated, and repurified by silica spin column chromatography (Qiagen; Qiagen). The first experiment characterized gene expression changes after CL treatment in B16 mice in EWAT of mice treated with CL for 0, 1, 3, and 6 days. The second experiment examined expression patterns in 129S1/SvImJ and 129S4/SvJae-Ppara<sub>wtGom</sub> mice treated with CL for 0, 3, and 6 days. (Results of 129S4/SvJae-Ppara<sub>wtGom</sub> mice are reported in Ref. 24a) This experiment also examined gene expression in mice treated with CL for 8 h. In this protocol, mice were injected with 30 nmol CL at the beginning of the experiment and 4 h later, and animals were killed 8 h after the first injection. Normalization of microarray signals and initial statistical analysis was performed by the University of Michigan Diabetes Research and Training Center using Affymetrix U74Av2 microarray. Microarray data have been deposited in the Gene Expression Omnibus under accession number GSE2130. Normalization of microarray fluorescence signals was performed, and initial statistical analysis was performed by the University of Michigan microarray core as detailed (http://dot.ped.med.umich.edu/2000/ourimage/pub/shared/Affymethods.html). Briefly, expression values from each experiment were normalized into 99 equal quantiles. Differences between control and drug-treated conditions were evaluated by the two-sided Wilcoxon signed-rank test, which evaluates perfect match vs. mismatch probe differences for each probe set. For clustering analysis of microarray data, we considered genes whose expression was 1.0 significantly changed vs. control (P < 0.01, two-sided Wilcoxon signed-rank test) at one or more time points, 2) significantly changed (P < 0.01) in both mouse B16 and 129S strains, and 3) up- or downregulated relative to control by a factor more than 2. We did not consider genes whose average quantified expression was <1.000 at all time points (i.e., genes whose expression was <2% of abundant genes at all time points).

Approximately 5% of genes on the microarray met these criteria. Normalized values from both experiments were averaged and evaluated by one-dimensional self-organizing map analysis (Expression NTI software; Informax). The biological relevance of identified clusters was evaluated by Onto-Express software using a hypergeometric distribution and false discovery rate correction (23, 24). Gene members of individual clusters or pooled clusters were evaluated as indicated in the text. Corrected values with a P < 0.01 were judged significant. Quantitative RT-PCR was performed as described (13).

RESULTS

Histological analysis. Adipose tissue of untreated mice contains a mixture of cell types that include mature unilocular adipocytes, vascular cells, and numerous small stromal cells. Nearly all mature fat cells contained a single large lipid storage droplet (LSD) and ranged in size from 30 to 50 μm (Fig. 1A). The histological appearance of white adipocytes after 1 day of CL treatment was similar to that of controls (Fig. 1B). However, several small regions of inflammatory cell (monocytes and macrophages) infiltration were observed, indicating local extravasation. The magnitude of this infiltration varied across animals and was most frequently observed near the proximal base of the epididymal pad.

The tissue showed pronounced signs of remodeling after 3 days of drug treatment (Fig. 1C). First, the size of the unilocular cells was significantly reduced. Numerous mature adipocytes exhibited strong fragmentation of the large LSD into numerous small lipid droplets that coincided with an increase in cytosolic eosin staining. By 3 days, many adipocytes had acquired a multilocular phenotype in which adipocytes had diameters >20 μm and contained at least eight well-defined lipid droplets that usually surrounded a central nucleus. Although these cells resembled brown adipocytes, nearly all were negative for expression of UCP1, a marker of brown fat cells (data not shown; also see Ref. 17).

Stromal cells remained <12 μm in diameter and in some cases appeared to have accumulated numerous very small lipid droplets. Some of these cells were organized into small patches, indicating that these cells are capable of migrating and perhaps proliferating. Finally, areas of inflammatory cell infiltration, particularly in the distal portions of the pad, were greatly reduced by 3 days of drug treatment.

EWAT was extensively remodeled after 6–7 days of CL treatment (Figs. 1D and 2). Hallmarks of this remodeling were extensive fragmentation of adipocyte LSD and strong cytoplasmic eosin staining. Stromal cells remained small (<12 μm), and many appeared to contain numerous small lipid droplets. CL-mediated remodeling was absent in mice lacking Adrb3, confirming the specificity of the compound (Fig. S1, supplemental data).

CL reduced the diameter of unilocular fat cells from 44 ± 1 to 31 ± 1 μm (P < 0.001) over the first 3 days, with no significant decline thereafter. The 30% decline in fat cell diameter approximates a 60% decline in cellular volume, reflecting the mobilization of triglyceride from these cells. Coinciding with the loss of lipid was the development of a multilocular phenotype. It is difficult to accurately determine the relative abundance of these cells in paraffin-embedded sections because processing of the tissue disrupts much of the fine structure of internal lipid droplets. However, techniques that allow imaging of intact adipocytes in adipose tissue indicate that ~85% of adipocytes had fragmented central LSDs after 3 days of CL treatment (14; also see Fig. 4C).

To examine whether changes in the cellular composition of EWAT involved proliferation, control and CL-treated mice were coinfused with BrdU to label cells undergoing DNA

References

replication. CL (for 3 or 7 days) greatly increased the mitotic index of cells within EWAT \( (P < 0.001) \) such that nearly 20\% of all nuclei were BrdU positive after 7 days of drug treatment (Fig. 3A). BrdU was incorporated in nuclei of stromal cells, multilocular adipocytes, and endothelial cells (Fig. 3B). After 7 days of continuous exposure to CL and BrdU, \( \sim 60-70\% \) of BrdU-positive cells were stromal, 25–30\% were multilocular adipocytes, and 3–6\% were endothelial cells.

The relative abundance of BrdU-labeled multilocular adipocytes increased over the course of drug infusion (Fig. 3C). To gain insight into the origin of the multilocular adipocytes, mice that had been treated with CL continuously for 3 days were injected two times with BrdU over a 6-h period. Although most cells labeled during this brief exposure to BrdU were stromal cells, numerous multilocular cells containing well-defined lipid droplets were labeled as well (Fig. 3D).

CL infusion for 6 days elevated the metabolic rate of EWAT in vitro in B6 mice (Fig. 4A). A similar increase in EWAT respiration was noted in congenic mice lacking Ucp1, the molecular effector of brown adipocyte thermogenesis (Fig. S2; supplemental data for this article may be found at http://ajpendo.physiology.org/cgi/content/full/00009.2005/DC1). Mitochondrial electron transport was monitored histochemically in B6 mice by observing the reduction of TTC to a red formazan product by tissue NADH oxidases. As shown in Fig. 4B, CL treatment greatly increased NADH-TTC oxidoreductase activity in whole EWAT pads. Last, we examined CL induction of mitochondria by confocal imaging of EWAT stained with MitoTracker Red. This technique, which images intact tissue, is superior to standard paraffin sections for imaging of mitochondria and lipid droplets in adipose tissue because of the greater depth of field achieved and the preservation of lipid droplet structure. Adipocytes of control tissue were nearly all unilocular and contained few mitochondria that were localized in the thin cytoplasm surrounding the major LSD (Fig. 4C). Six days of CL treatment fragmented the major LSD of most fat cells and strongly elevated MitoTracker staining in the space between the lipid droplets.

Expression profiling analysis. The histological and metabolic data indicate that CL treatment alters the cellular and
Fig. 3. Effect of CL on bromodeoxyuridine (BrdU) incorporation in EWAT. A: total mitotic index in EWAT of mice coinfused with CL and BrdU for 3 or 7 days. Controls were infused with BrdU alone for 7 days (n = 6 animals). B: BrdU immunohistochemistry showing numerous multilocular adipocytes containing BrdU-positive (BrdU+) nuclei (arrows). Nuclei of BrdU-positive adipocytes were defined by surrounding internal lipid droplets. *BrdU-positive adipocyte with partially fragmented lipid droplet. C: mitotic index of multilocular adipocytes identified in paraffin sections (n = 6). D: incorporation of BrdU into nuclei of multilocular adipocytes in EWAT after 6 h of exposure to BrdU. CTL, control. Bar = 25 μm.

Fig. 4. CL treatment increases oxidative capacity of EWAT and induces mitochondrial biogenesis. A: respiration rate of EWAT minces of B6 control mice and mice treated with CL for 6 days measured in vitro using polarographic electrodes (n = 3–7). B: histochemical detection of 2,3,5-triphenyltetrazolium chloride (TTC) reductase activity of whole EWAT pads of B6 control and CL-treated mice. C: EWAT of B6 control (left) and CL-treated (right) mice stained with Mitotracker Red and imaged by laser scanning confocal microscopy.
metabolic character of WAT. To gain insight into the mechanisms involved, we assessed the patterns of gene expression over the course of tissue remodeling using microarray analysis.

Two independent experiments were performed using Bl6 and 129S mice, and genes meeting the criteria specified in METHODS were evaluated by self-organizing map-clustering analysis. Six-hundred fifty of ~12,000 genes present on the array met criteria for clustering. The aim of the global profiling was to assess underlying biological processes involved in adipose tissue plasticity rather than evaluating the significance of individual genes. Therefore, the biological relevance of these clusters was evaluated using Onto-Express software, a statistical tool that calculates the likelihood that genes of defined functions would be enriched in particular clusters (23, 24).

Figure 5 is a visual representation of nine clusters identified by self-organizing map analysis, arranged by cluster size. The few genes in clusters 1 and 2 demonstrated somewhat erratic expression patterns, had diverse gene ontologies, and were not considered further. In general, three major temporal expression patterns emerged in the remaining clusters. Genes in clusters 4–7 had low basal expression and were sharply, but transiently, induced by CL treatment. Genes in clusters 8–9 were transiently downregulated by CL, with genes in cluster 9 fully recovering expression, whereas those in cluster 8 remaining somewhat downregulated. Genes in cluster 3 were initially suppressed and then induced to levels beyond that of untreated mice.

Transiently induced genes (clusters 4–7) constituted 45% of the genes meeting the criteria for clustering. The individual clusters differed with respect to peak and duration of induction, with genes in cluster 7 showing immediate transient induction while genes in cluster 5 were somewhat delayed. Genes in cluster 4 were immediately induced, and upregulated expression endured over 3 days of drug exposure. GO analysis of the transiently induced clusters (i.e., clusters 4–7) indicated significant enrichment for genes involved in inflammation (GO: 0006954, \( P = 4.4 \times 10^{-11} \)), innate immune response (GO: 0006955, \( P = 7.9 \times 10^{-6} \)), and chemotaxis (GO: 0006925, \( P = 9.9 \times 10^{-8} \)). Together, clusters 6 and 7 were particularly enriched in genes involved in the production and transduction of cytokine signals (GO: 0005125, \( P = 7.9 \times 10^{-6} \)), whereas genes of cluster 5 were significantly associated with the cell cycle (GO: 007049, \( P < 0.0001 \)) and proliferation (GO: 008283, \( P < 0.003 \)). Also within cluster 7 were genes likely to be engaged in response to the excessive mobilization of free fatty acids and glycerol, including glycerol kinase, fatty acid CoA ligase-4, adipose differentiation-related protein, and carnitine palmityltransferase-1a.

Genes that were transiently suppressed by CL treatment (clusters 8 and 9) constituted 42% of genes meeting clustering criteria and included a range of ontology categories, including fatty acid metabolism (GO: 000653, \( P < 0.007 \)), carboxylic acid metabolism (GO: 0008152, \( P < 1 \times 10^{-6} \)), and cell adhesion (GO: 007155, \( P < 1 \times 10^{-5} \)). Interestingly, genes that are known to be selectively or exclusively expressed in adipocytes are heavily represented in these clusters, including those involved in lipogenesis (Glut4/Slc2a4, fatty acid synthase, malic enzyme, and lipoprotein lipase), lipolysis (hormone-sensitive lipase and Adrb3), and endocrine function (adiponectin and leptin). It is notable that the cluster also contains peroxisome proliferator-activated receptor-\( \gamma \) (Pparg) and CAAT enhancer-binding protein-\( \alpha \) (Cebp\( \alpha \)), two transcription factors known to play a central role in fat-specific gene expression (GO: 0045444, \( P = 2.6 \times 10^{-9} \)).

The third major expression pattern contained genes that were initially suppressed but then were overexpressed by 6 days of treatment (cluster 3). Thirty-six of the 43 genes in this cluster...
are annotated to some degree while the remainder currently have unknown functions. Inspection of the annotated genes in this cluster indicated 23 of 36 are either nuclear genes targeted to mitochondria or play a direct role in mitochondrial metabolism in general, and, specifically, in the transport, activation, and oxidation of fatty acids (Fig. 5). Statistical analysis of this cluster indicated significant representation of genes involved in the electron transport (GO: 0006118, \( P = 1.2 \times 10^{-5} \)), mitochondrial inner membrane (GO: 0019866, \( P = 4.9 \times 10^{-7} \)), and the catabolism of free fatty acids (GO: 0006631, \( P = 9.9 \times 10^{-8} \)). Interestingly, the only transcription factor represented in this cluster was peroxisome proliferator activated receptor-\( \alpha \) (Ppara), a nuclear receptor of fatty acids that is known to be involved in mitochondrial biogenesis and fatty acid oxidation (8, 16).

**DISCUSSION**

The present results indicate that sustained activation of Adrb3 induces pronounced cellular and metabolic plasticity in WAT. Histological and gene profiling data indicate that prominent features of CL-induced plasticity include 1) a transient "inflammatory" response, 2) proliferation of stromal cells and multilocular adipocytes, and 3) induction of mitochondrial biogenesis and of \( \beta \)-oxidation in multilocular adipocytes. As developed below, the overall pattern of gene expression and tissue remodeling suggests that CL-induced plasticity is a coordinated homeostatic response that is driven by the excessive efflux of fatty acids (Fig. 6).

The histological and molecular data indicate that Adrb3-induced remodeling involves three temporally distinct processes. First, CL treatment induced a rapid, but transient, inflammatory response. Although the signals inducing the inflammatory response were not identified, it is likely that mobilized free fatty acids are involved. Free fatty acids activate proinflammatory signaling in numerous cell types (3, 4, 22, 35), and the inflammatory response in adipose tissue was temporally correlated with the bulk of fatty acid mobilization, which occurred during the first 3 days of drug treatment. Interestingly, the inflammatory response waned when the capacity of the tissue to oxidize fatty acids increased. Furthermore, animals with impaired ability to expand mitochondrial biogenesis or to oxidize fatty acids had less 

![Fig. 6. Working model for CL-induced adipose tissue remodeling. Top: CL-induced remodeling involves temporally distinct processes. CL treatment induces an immediate, but transient, inflammatory response that includes release of chemotaxic chemokines, recruitment of circulating myeloid cells, and remodeling of the extracellular matrix (ECM). During remodeling, CL induces cellular proliferation and migration among stromal cells and division of some multilocular adipocytes. Mature fat cells become multilocular and expand oxidative capacity by generating new mitochondria and increasing expression genes that oxidize fatty acids. FFA, free fatty acid. Bottom: Quantitative RT-PCR (qRT-PCR) analysis of mRNA levels of genes representative of inflammation, mitogenesis, and mitochondrial biogenesis. RNA from EWAT of individual C57BL/6 (B6) mice was evaluated by qRT-PCR (\( n = 4–9 \)).](http://ajpendo.physiology.org/ by 10.220.32.246 on October 21, 2017)
oxidative capacity have a greatly exaggerated inflammatory response to CL treatment (24a). Together, these observations indicate that mobilized fatty acids promote local inflammatory responses and that expansion of oxidative capacity in WAT limits that response.

The transient inflammatory response was inversely correlated with the suppression of adipocyte-specific gene expression. Inflammatory cytokines like tumor necrosis factor-α suppress phenotypic expression of model adipocytes in vitro, and this suppression is correlated with loss of Cebpa and Pparg (19, 31, 38). Transient inhibition of adipocyte phenotypic markers during CL treatment was correlated with the transient loss of Cebpa and Pparg mRNA, raising the possibility similar mechanisms exist for CL-induced inhibition of adipocyte phenotypic expression.

The overall inflammatory response likely involves interactions among resident cells and recruitment of circulating inflammatory cells. Recent studies indicate that both adipocytes and stromal cells can express proinflammatory cytokines like monocyte chemotactic protein-1 and macrophage inflammatory protein-γ (29), which are likely to contribute to extravasation, cell migration, and tissue remodeling. Although the pattern of inflammatory gene expression is similar to that triggered by pathogens, inflammatory genes are often induced during normal development and physiological remodeling (1, 9, 34). A key feature of the inflammatory response is the expression of genes that remodel the extracellular matrix (ECM). Clearly, cell proliferation and migration require reorganization of the ECM, and recent work indicates that ECM remodeling might be sufficient to promote adipogenesis under certain conditions (1, 21, 25). As in other systems, adipose tissue stromal cells are likely to play a key role in tissue remodeling during catabolic stress. Recent work indicates that adipose tissue stromal cells express numerous genes that are typically associated with macrophages, and the term ‘adipo-phage’ has been coined to describe the developmental plasticity of these cells (6).

A second major feature of CL-induced remodeling is cellular proliferation. Inspection of cell cycle gene expression indicates that mitogenic activity lags behind inflammatory gene expression, peaks around the 3rd day of treatment, and drops off somewhat by day 6. Although most of the BrdU-positive cells in adipose tissue were stromal cells, ∼20–30% of the BrdU-positive cells were multilocular, which represented up to 20% of the total population of multilocular cells. The time course of histological changes strongly indicates that multilocular cells are derived from mature adipocytes that have undergone progressive fragmentation of the main LSD. Indeed, multilocular cells could be labeled during a 6-h exposure to BrdU, providing evidence that lipid-laden adipocytes can divide during catabolic stimulation. Although this phenomenon has not been previously observed in vivo, several reports have demonstrated the proliferative potential of lipid-laden adipocytes in vitro (32, 33). Interestingly, the ability of fat cells to divide in vitro appears to require fragmentation of the central lipid droplet, and this process is accelerated by catabolic stimulation. The present data suggest as much as 20% of the population of multilocular fat cells could be derived from adipocyte cell division. An alternative, that multilocular cells originated from the recruitment of stem cells, seems unlikely because this would require cell division, differentiation, and accumulation of lipid to a cell diameter >20 μm in 3 days in the face of ongoing lipolytic stimulation. Previous analysis of CL-induced remodeling in rat WAT reported a pronounced increase in BrdU labeling that was nearly exclusively limited to endothelial cells, although rare BrdU-positive multilocular cells were observed as well (17). In contrast, we found significant incorporation of BrdU in nuclei of stromal cells and multilocular adipocytes. The reasons for this difference are presently unclear but could be related to methodological differences, including dose, duration, and species. We have observed pronounced CL-induced BrdU incorporation in rats and in five mouse strains (unpublished observations), indicating that this is a fundamental component of tissue remodeling.

A third major feature of CL-induced tissue plasticity is the pronounced expansion of catabolic capacity that includes stimulation of mitochondrial biogenesis and elevated oxidative metabolism. The mitochondrial biogenesis was restricted to large multilocular adipocytes and was strongly correlated with the induction of fatty acid oxidation genes. Induction of mitochondrial biogenesis in white adipocytes does not require systemic activation of Adb3 receptors but can be observed in individual white adipocytes transduced in vivo with constitutively activated β-adrenergic receptors (14). The upregulation of genes that transport, activate, and oxidize fatty acid within adipose tissue can be viewed as an adaptive mechanism that would minimize systemic release of fatty acids. In this regard, chronic (but not acute) treatment with Adb3 agonists lowers circulating free fatty acid levels (20, 30). Interestingly, the patterns of proinflammatory and adipocyte-specific gene expression were inversely correlated, and both patterns were normalized when catabolic activity of WAT increased. These observations are consistent with the hypothesis that excessive fatty acid mobilization induces a proinflammatory state that suppresses the anabolic functions of adipocytes, and that homeostatic activation of fatty acid catabolism restores adipose tissue function, in part by reducing proinflammatory signals.

The biochemical basis for elevated WAT respiration is not entirely understood. Analysis of gene expression patterns indicates that ATP-consuming “futile” substrate cycling could play a role in elevated metabolism. For example, pathways involved in lipid synthesis return to normal after 6 days of drug treatment, at a time in which genes involved in fatty acid catabolism are strongly upregulated. This pattern of gene expression is similar to that seen in thermogenically active brown fat (37). Inspection of the gene profiling results suggests that cycles of fatty acid hydrolysis/esterification (Lipe/glycerol kinase/diacylglycerol acyltransferase-1) and enoyl-CoA reduction/oxidation (nuclear receptor binding factor-1) could contribute elevated metabolism of WAT (18, 27). It is also possible that uncoupling of oxidative phosphorylation contributes to the elevated metabolic rate. Fatty acids, which are mobilized by CL, are potent mitochondrial uncouplers in numerous cell types (36). Whether specific Ucops play a role in elevated EWAT oxidative metabolism is not presently known. Ucp1–3 were present on the array; however, expression of Ucp2 and -3 was not great enough to meet the criteria for clustering. Ucp1 was expressed in WAT at very low levels and was induced by CL treatment. The magnitude and pattern of Ucp1 expression differed greatly between mouse strains (also see Refs. 5 and 15), and even maximal induction was <3% of levels in BAT (Granneman, unpublished observation). Neither the elevation
of EWAT metabolic rate nor the induction of mitochondrial biogenesis and lipid oxidation genes require Ucp1 (present study and Ref. 13). Importantly, Ucp1 did not cluster with other mitochondrial genes (cluster 3), and its induction likely represent events that are distinct from the mitochondrial biogenesis that takes place in white adipocytes. Taken together, these observations indicate that Ucp1 expression is not a necessary or consistent feature of CL-induced adipose tissue remodeling.

The present experiments focused on remodeling in EWAT. Although we have observed qualitatively similar remodeling in inguinal, retroperitoneal, and suprascapular white fat pads, these depots are heterogeneous in their responsiveness to physiological and pharmacological stimuli. For example, EWAT appears to be least capable of inducing Ucp1 expression and thus is a good choice for analysis of Ucp1-independent processes. Nonetheless, it is likely that the magnitude of remodeling and the impact of recruited brown adipocytes on tissue function could vary among fat pads.

In summary, chronic pharmacological activation of Adbrb3 remodels WAT by stimulating cell proliferation and augmenting catabolic capacity. Whether Adbrb3 agonists will prove useful in humans is debatable given the low levels of receptor found in human fat (12). Nonetheless, there is growing evidence that metabolic and cellular remodeling of adipose tissue can have a large effect on systemic insulin sensitivity, and an understanding of the mechanisms involved could lead to new avenues of therapeutic intervention.

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