Role of hormone-sensitive lipase in β-adrenergic remodeling of white adipose tissue

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Mottillo EP, Shen XJ, Granneman JG. Role of hormone-sensitive lipase in β-adrenergic remodeling of white adipose tissue. Am J Physiol Endocrinol Metab 293: E1188–E1197, 2007. First published August 21, 2007; doi:10.1152/ajpendo.00051.2007.—Free fatty acids (FFA) are important extracellular and intracellular signaling molecules. We previously hypothesized that the acute and chronic responses to β3-receptor stimulation in white adipose tissue (WAT) are adaptations to the excessive efflux of FFA (25). In this model, the mobilization of FFA in WAT triggers an acute inflammatory response, which wanes over time as fat cells expand mitochondrial mass and upregulate genes involved in FFA oxidation through the action of the peroxisome proliferator-activated receptors (PPARs).

Adipose tissue is the major site of TG storage and catabolism, wherein FFA are released via β-adrenergic-stimulated lipolysis. Lipolysis is triggered by the release of multiple lipases (reviewed in Ref. 1). Adipose triglyceride lipase (ATGL) (10, 36) and patatin-like phospholipase domain containing 2, is a newly discovered lipase that acts on both TG and diacylglycerol (DAG), and is highly dependent on the presence of calcium in the cytosol. ATGL activity appears to be indirectly regulated by protein kinase A (PKA) activation through the phosphorylation-dependent release of its coactivator α/β hydrolase domain containing protein 5 (Abhd5) from perilipin (8, 35). Hormone-sensitive lipase (HSL) acts on both TG and diacylglycerol (DAG), and phosphorylation of HSL by PKA selectively increases its TG hydrolase activity (reviewed in Ref. 20). Last, monoacylglycerol lipase catalyzes the final breakdown into glycerol and FFA.

To investigate the role of FFA in β3-AR-induced tissue remodeling, we used genetic and pharmacological inactivation of HSL to reduce FFA mobilization during β3-AR activation. HSL can hydrolyze cholesteryl esters and retinyl esters (20), although its main function in adipose tissue is to act as a triglyceride/diglyceride lipase (17, 32). Consistent with its role as a major lipase, genetic deletion of HSL reduces circulating FFA levels and cuts β-adrenergic-stimulated lipolysis in half (11, 29, 34). In addition, deletion of HSL promotes adipocyte hypertrophy but does not result in obesity, possibly due to increased fat cell apoptosis (13, 29, 34). Our results indicate that genetic or selective pharmacological inhibition of HSL prevents upregulation of inflammatory cytokine expression by β-AR activation, supporting a role of mobilized FFA as signaling molecules in adipose tissue. The effects of β3-AR activation on inflammatory signaling, however, were not affected in mice with genetic disruption of Toll-like receptor 4 (Tlr4) signaling, indicating that inflammation is not a mediator of β3-AR activation.
delayed in HSL-knockout (KO) mice. The results indicate that HSL-mediated lipolysis and subsequent downstream FFA signaling are important components of adipose tissue remodeling following acute and chronic β3-AR activation.

METHODS

Animals, surgery, and drug treatment. HSL-KO mice (29) were supplied by Dr. F. Kraemer (Stanford University). HSL+/− mice on a C57Bl/6 background were bred at Wayne State University. Mice were genotyped by PCR using primers HSL-A, 5′AGAGAGCAACCT-CAGGCTTCCAC-3′; HSL-B, 5′CAAAGTTGTCTGTGCTGT-TGTCTT-3′; and SI-166, 5′AGATTGAAACAGATACGCA-3′; where the WT allele corresponds to a 269-base pair (bp) band and the mutant allele to a 320-bp band. Heterozygous (Het) mice were phenotypically identical to WT mice (34) and displayed similar levels of inflammatory markers to that of WT mice (data not shown). Therefore, Het mice were grouped with WT as controls for analysis where indicated. Mice with inactivated Tlr4 (Tlr−/−) and WT congenic controls (BALB/cAnPt) were obtained from Jackson Laboratories. All mice were weaned at 3 wk of age and fed rodent chow (Test Diet, no. 5001) and tested when 8–9 wk old. Both male and female mice were used in experiments. Experimental procedures involving animals were approved by the Wayne State University Animal Investigation Committee.

To examine the effects of β3-AR stimulation on acute inflammatory responses, WT and HSL-KO mice were injected intraperitoneally with 10 nmol of CL and killed 6 h later. To examine the effects of pharmacological inhibition of HSL, WT mice (n = 7–8) were pretreated with 30 mg/kg of the selective HSL inhibitor BAY 59-9435, also known as 4-isopropyl-3-methyl-2-[1-[3-(S)-methyl-piperidin-1-yl]-methanoyl]-2H-isoxalo-5-one (BAY), and suspended in 0.5% methylcellulose or methylcellulose alone via oral gavage (5). After 1 h, mice were injected intraperitoneally with 10 nmol of CL or H2O and killed 3 h later, which encompassed the maximal effect of BAY and maximal expression of chemokine ligand 2 (Ccl2; data not shown). Epididymal white adipose tissue (EWAT) pads were removed and processed for mRNA analysis as described below. In experiments with Tlr−/− and WT BALB/c mice, acute CL treatment lasted 3 h.

To examine the effects of chronic β3-AR activation, mice were anesthetized with halothane and implanted with osmotic minipumps (C else, microvascular needle attached with heparinized saline) and emulsified in 0.5% methylcellulose or methylcellulose alone via oral gavage. The next morning, medium was replaced with serum-free, phenol red-free, DMEM-F-12 medium described (28), with where indicated. Mice with inactivated Tlr4 (Tlr−/−) and WT congenic controls (BALB/cAnPt) were obtained from Jackson Laboratories. All mice were weaned at 3 wk of age and fed rodent chow (Test Diet, no. 5001) and tested when 8–9 wk old. Both male and female mice were used in experiments. Experimental procedures involving animals were approved by the Wayne State University Animal Investigation Committee.

Tissue fractionation and immunoblotting. EWAT was homogenized in HEPES-EDTA-sucrose (HES; 10 mM HEPES, pH 7.8, 250 mM sucrose, 1 mM EDTA) and centrifuged at 500 g for 10 min to create a postnuclear supernatant. The postnuclear supernatant was then centrifuged again at 18,000 g for 25 min to create a crude mitochondrial pellet and crude cytosol. The mitochondrial pellet was then resuspended in HES, vortexed, and sonicated briefly on ice, and protein quantification was performed using the bicinchoninic acid method (Pierce).

Antibodies used were prohibitin (Abcam, ab2996, diluted 1:1000) and nuclear receptor binding factor-1 (NRF1; diluted 1:1000). SDS-PAGE was performed under standard conditions using 10% minigels (Invitrogen). Resolved proteins were transferred to polyvinylidene difluoride, and membranes were blocked for 1 h at room temperature in 5% powdered skim milk, probed with respective secondary antibodies, and visualized using the Supersignal chemiluminescent kit (Pierce).

Adipose tissue was fixed and embedded in paraffin as detailed previously (25). Mitochondrial electron transport activity of VAT was determined by measuring the reduction of 2,3,5-triphenyltetrazolium chloride in tissue explants (7). Adipocyte mitochondria were stained in whole mount adipose tissue with streptavadin-Alexa 555 and imaged by spinning disc confocal microscopy (25).

mRNA expression. For analysis of mRNA, EWAT was placed in RNA later (Ambion) and held at −80°C until processed. RNA from EWAT was extracted with Trizol (Invitrogen) and then purified with a RNaseasy mini kit (Qiagen). Twenty to fifty nanograms of cDNA was subjected to qPCR analysis, using SYBR Green as the fluorescent reporter, and expression levels were normalized to that of peptidylprolyl isomerase A mRNA. PPARα cDNA was amplified using forward and reverse primers 5′-CTAAACCTGGG-CACACT-3′ and 5′-CCGGTACCTGAGTCTGTA-3′, respectively, and matrix metalloproteinase 12 (MMP12) cDNA was quantified with 5′-GAGCTCATGGACCCGTCTT-3′ and 5′-ACGCAG-CAGGGGTCATT-3′. All other qPCR primers were previously described (25).

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primary antibodies at room temperature for 1 h, incubated with a secondary goat anti-rabbit horseradish peroxidase (diluted 1:3,000; Cell Signal Technologies), and quantified using a Bio-Rad Quantity One imaging system.

Statistical analysis. Data were evaluated by one- or two-way ANOVA. Post hoc comparisons were performed using the Bonferroni t-test. Two-tailed t-tests were performed where indicated.

RESULTS

HSL-KO mice are refractory to the acute induction of inflammation by CL. Infusion of CL triggers a transient inflammatory response in WAT that involves elevated expression of chemotactic chemokines and the recruitment and extravasation of myeloid cells (7). In the present work, we found that a single injection of CL was sufficient to provoke the expression of proinflammatory cytokines Ccl2 and Ccl9 in WT and Het mice (Fig. 1). CL treatment slightly elevated the expression of the macrophage marker EMR1 (also known as F4/80) over this time frame but did not increase low levels of the macrophage-specific MMP12. HSL-KO mice had significantly elevated expression of all proinflammatory markers in the unstimulated state, including pronounced elevation of the macrophage markers EMR1, as previously reported (4, 12), and MMP12. Acute CL treatment did not trigger a further increase in the expression of these markers.

Histological examination showed pronounced differences in the appearance of WT and KO WAT. Adipose tissue of WT mice is a mixture of adipocytes and stromal cells that contain very few macrophages and monocytes in the basal state (Fig. 2A). In contrast, HSL-KO mice exhibited numerous “crown structures” (4) in unstimulated WAT (Fig. 2C). These structures contain activated macrophages, as evidenced by intense F4/80 staining (4, 12), and have been hypothesized to be localized sites of adipocyte necrosis and clearance (4). Injection of CL triggered pronounced myeloid cell recruitment and extravasation within EWAT of WT mice and heterozygous mice (Fig. 2B). CL had no additional effect on HSL-KO mice, nor did mice at any time demonstrate myeloid cell recruitment and extravasation patterns seen in WT/Het mice (Fig. 2D).

Pharmacological inhibition of HSL prevents induction of inflammatory cytokines in vivo and in vitro. The above results suggest that myeloid cell recruitment by CL requires HSL. Nonetheless, the results are potentially complicated by the persistent upregulation of inflammatory markers associated with activated macrophages in WAT of HSL-KO mice. To address this issue we examined the effects of BAY, a potent and selective pharmacological inhibitor of HSL (5, 21, 26), on CL-induced inflammation.

BAY has previously been shown to be inactive on several lipases, including ATGL (21, 26), and has been reported to have no effect on the residual lipase activity seen in HSL-KO mice (21). However, these data are not entirely unequivocal since they did not test ATGL that was activated by its coactivator Abhd5 (22), nor did they examine HSL-KO mice under conditions where ATGL activity could be observed (21). Therefore, we examined the effects of BAY on FFA and glycerol efflux from adipocytes of WT and HSL-KO mice as well as the effects on recombinant ATGL and HSL.

Fig. 1. Effect of acute CL-316,243 (CL) on mRNA levels of inflammatory markers. Effect of acute CL on epididymal white adipose tissue (EWAT) mRNA levels of chemokine ligand 2 (Ccl2), chemokine ligand 9 (Ccl9), EMR1 (also known as F4/80), and matrix metalloproteinase 12 (MMP12) of wild-type (WT)/heterozygote (Het) and hormone-sensitive lipase-knockout (HSL-KO) mice (n = 8–11). One-way ANOVA indicates a significant drug effect (\*P < 0.05) and a significant genotype effect (#P < 0.05; ##P < 0.01; ###P < 0.001). Two-tailed t-test shows a significant difference between HSL-KO control (Ctl) and CL treatment vs. WT/Het Ctl for Ccl2. Two-tailed t-test (&) indicates a significant drug treatment effect for EMR1 in WT/Het animals. PPIA, peptidylprolyl isomerase A.
In WT adipocytes, BAY pretreatment reduced CL-induced FFA efflux by 55% (Fig. 3A) and virtually eliminated glycerol release (Fig. 3B). As expected, deletion of HSL reduced, but did not eliminate, CL-stimulated FFA efflux (Fig. 3A) while virtually abolishing induced glycerol release (Fig. 3B). Importantly, BAY had no effect on residual FFA or glycerol efflux in HSL-KO animals, demonstrating the molecular specificity of BAY. To test whether BAY affects ATGL activity stimulated by its coactivator Abhd5 (22), we looked at the effects of BAY on recombinant HSL, ATGL alone, and Abhd5-ATGL in an in vitro assay. As expected, BAY eliminated HSL-dependent lipase activity (Fig. 3C). ATGL exhibited little lipase activity on its own, whereas incubation with Abhd5 increased its activity to ~50% of HSL activity. BAY had no significant effect on ATGL alone or in the presence of Abhd5.

With the specificity of BAY clearly demonstrated, we treated mice with BAY to study the role of HSL in the acute response to CL. Acute pharmacological inhibition of HSL via BAY had no effect on inflammatory cytokine Ccl2 expression in unstimulated WAT (Fig. 4A). Induction of proinflammatory Ccl2 by CL, however, was nearly completely prevented by chemical inhibition of HSL. Along with data from KO mice, these data strongly indicate that CL induction of WAT inflammation requires HSL activity.

We next examined whether β-AR activation was sufficient to upregulate inflammatory gene expression in cultured adipocytes and whether this effect required HSL activity. Figure 4C shows that, as expected, isoproterenol strongly elicited mobilization of FFA from 3T3-L1 adipocytes, and this effect was sharply attenuated by inhibition of HSL with BAY. Selective β-AR stimulation of 3T3-L1 adipocytes significantly elevated expression of Ccl2, and this effect was completely abolished by pretreatment with BAY (Fig. 4B). Acute pharmacological inhibition of HSL had no effect on Ccl2 expression in unstimulated adipocytes.

Induction of proinflammatory signaling by CL does not require Tlr4 signaling. The above data indicate that HSL-dependent mobilization of FFA acutely upregulates proinflammatory cytokine expression in white adipocytes. The nature of the mechanism(s) that detects mobilized FFA is not known; however, it has been suggested that the Tlr4 is an FFA receptor that is capable of triggering proinflammatory responses like those induced by CL (23, 24, 31). To examine the role of Tlr4, we challenged WT mice and congenic mice with disrupted Tlr4 signaling with CL. As shown in Fig. 5, Tlr4-defective mice had extremely low levels of proinflammatory markers in the unstimulated state. Nonetheless, CL induced these markers to an extent that equaled or exceeded that seen in WT mice.

Induction of mitochondrial oxidative capacity by CL is significantly delayed in HSL-KO mice. Chronic stimulation of β3-AR triggers mitochondrial biogenesis, induces expression of genes involved in fatty acid oxidation, and elevates tissue metabolic rate (25). Previous work demonstrated that the nuclear receptor PPARα is essential for this process, and we hypothesized that FFA might be important endogenous agonists of PPARα during β3-AR simulation. In WT/Het mice, infusion of CL for 3 days reduced fat cell size and strongly fragmented the central lipid droplet of adipocytes such that most cells acquired a multilocular appearance with enhanced eosin staining (indicative of mitochondria) between the fragmented droplets (Fig. 6). In contrast, HSL-KO mice showed virtually no signs of adipocyte remodeling after 3 days of CL infusion, and cells retained their unilocular appearance. After 7 days of CL treatment, EWAT remodeling was further pronounced in WT/Het mice, with greater eosin staining and an increased degree and number of multilocular fat cells. By 7 days of CL, WAT of HSL-KO mice exhibited signs of lipid droplet fragmentation and elevated eosin staining.

We next examined the importance of HSL on the regulation of genes involved in mitochondrial biogenesis and oxidative capacity (Fig. 7). Expression of PPARα was similar between
Both genes were expressed at similar levels under control conditions and were sharply upregulated at 3 days in control mice but not in KO mice. However, by 7 days of CL, expression of these genes in KO mice was similar to controls. Immunoblot analysis confirmed that, by 7 days of CL, the mitochondrial markers prohibitin and NRBF1 were similarly upregulated in both genotypes (Fig. 8, A and B), as was the ability to reduce triphenyltetrazolium, a marker of mitochondrial electron transport activity. We also examined the appearance and distribution of mitochondria in fixed whole mount fat pads by fluorescence confocal microscopy, using streptavidin-Alexa 555 to selectively label mitochondria. Virtually all genotypes under control conditions. After 3 days of CL infusion, PPARα mRNA levels were upregulated fourfold in WT/Het mice but were unchanged in HSL-KO mice. However, by 7 days of CL, expression of these genes in KO mice was similar to controls.
Mitochondrial staining was associated with mature adipocytes, with little or no staining observed in stromal cells (Fig. 8D). Under control conditions, adipocyte mitochondrial staining was sparse, with heaviest concentrations immediately surrounding the nucleus. After 7 days of CL treatment, nearly all WT cells assumed a multilocular morphology, with intense streptavidin staining surrounding the fragmented lipid droplets. Adipocytes of KO mice also exhibited pronounced elevation of mitochondrial staining; however, because of the relative lack of lipid droplet fragmentation, the expanded mitochondrial staining was concentrated in the thin cytoplasmic space between the core lipid droplet and the plasma membrane. Interestingly, adipose tissue of CL-treated HSL-KO mice remained inflamed, as indicated by the macrophage marker EMR1, despite expanded oxidative capacity (Fig. 8E).

**DISCUSSION**

Chronic stimulation of β3-AR remodels white adipose tissue and produces a cellular phenotype with expanded mitochondrial mass, upregulated fatty acid oxidation gene expression, and elevated metabolic rate (6, 14). Analyses of this metabolic plasticity indicate that β3-AR stimulation engages two interrelated physiological events: transient inflammation, which occurs at the onset of drug treatment, followed by expansion of oxidative capacity that takes place over several days (7, 25). We hypothesized that tissue remodeling is an adaptive response to the excessive efflux of fatty acids provoked by β3-AR stimulation (9, 14, 25). In this model, excessive FFA induce adipose tissue inflammation, which is countered over time by the expanded capacity of the fat cells to oxidize lipid. Aside from the reciprocal temporal relationship between inflammation and oxidative capacity, the model is supported by the fact that PPARγ knockout mice, which are incapable of expanding mitochondrial oxidative capacity during β3-AR stimulation, exhibit sustained inflammation during CL treatment (25). In this model, mobilized FFA function as signaling molecules that trigger inflammation and activate PPARγ, which control expansion of mitochondrial oxidative capacity.

Recent work (8, 35) indicates that PKA-dependent lipolysis in fat cells involves the complementary activities of ATGL and HSL. Hormone-sensitive lipase is a major triglyceride/diglyceride lipase in adipose tissue, and its inhibition substantially reduces PKA-stimulated mobilization of FFA. Although fat cell FFA production is not entirely dependent on HSL, genetic

![Graph A](image1)

![Graph B](image2)

**Fig. 5.** Effect of acute CL (10 nmol) on inflammatory markers Ccl2 and Ccl9 in BALB/cAnPt (BALB/c) and Toll-like receptor 4 (Tlr4Lps-d) mice. Two-way ANOVA indicates a significant CL effect (P < 0.01) for both Ccl2 and Ccl9.

**Fig. 6.** Effect of chronic CL infusion on the histological appearance of EWAT. Shown are representative H&E-stained sections of EWAT of WT/Het and HSL-KO mice after 3 and 7 days of control or CL treatment. Bar = 100 μm.
PKA activation of HSL leads to increased hydrolytic production of FFA, and FFA are known to be proinflammatory mediators in several cell types (1, 2). HSL can hydrolyze cholesterol and retinyl esters as well (20); however, the magnitude to which this occurs in fat cells in response to PKA activation is not clear, nor is it clear whether the possible products (free cholesterol and retinol) are inflammatory mediators. Similarly, it is unlikely that ATGL-mediated production of DAG is involved in the acute effects of CL since BAY reduced inflammation rather than increasing it, as would be expected if DAG were the proinflammatory mediator.

We were surprised to find that inflammatory gene markers were persistently elevated in adipose tissue of HSL-null mice, a result that was reported while this work was in progress (4, 12). Interestingly, CL challenge did not increase expression of inflammatory markers in knockout mice, nor did it lead to extravasation of immune cell sites observed in wild-type mice, suggesting distinct mechanisms for the inflammation. These observations indicate that HSL is required to increase inflammatory gene expression after acute CL challenge and are consistent with the hypothesis that CL-induced inflammation requires local generation of FFA. Surprisingly, the persistent inflammation observed in EWAT of the HSL-KO animal did not abate under chronic CL administration as it does in wild-type mice (7, 25). These results indicate that reduction in cell size and elevation of FFA oxidation are not sufficient to normalize inflammation in the HSL-KO mice.

The mechanism of persistent WAT inflammation in HSL-KO mice is currently not understood and could be due to any of several signaling events. HSL-KO mice have clear foci of activated macrophages that are likely to be a major source of inflammatory gene expression (4). It is possible that macrophage recruitment in HSL-null mice involves the chronic loss of anti-inflammatory products like retinoic acid (12) or the accumulation of proinflammatory substrates like DAG. Regardless, the chronic inflammation in HSL-KO mice differs in several respects from that triggered acutely by CL, and these almost certainly involve distinct signaling pathways.

The discordance between genetic and pharmacological inhibition of HSL with regard to levels of inflammatory cytokines is likely related to acute vs. chronic/developmental inhibition. Pharmacological inhibition with BAY occurred over a short period, whereas the KO the animal is defective for HSL throughout development. Although promising as an acute anti-inflammatory therapeutic, additional experiments are needed to assess the long-term effects of BAY.

It is uncertain how mobilized FFAs are detected so as to trigger an inflammatory response. Tlr4 has been proposed (31) to be a receptor that links excessive FFA to innate inflammatory responses. We found that basal levels of inflammatory markers were extremely low in mice with deficient Tlr4 signaling, consistent with a role of Tlr4 inflammatory signaling in adipose tissue. Nonetheless, the induction of inflammatory markers by CL was fully intact in Tlr4-defective mice, indicating that Tlr4 is not required to link mobilized FFA to CL-induced inflammatory responses. Our results do not exclude the existence of another FFA sensor/receptor but suggest that molecules linked to FFA metabolism, such as ceramide (15) or DAG, lead to the activation of other pathways, such as atypical protein kinase C isoforms (16).

**Fig. 7.** Induction of mitochondrial biogenesis and β-oxidation is delayed in HSL-KO mice. Effect of chronic CL on mRNA expression of peroxisome proliferator-activated receptor-α (PPARα), cytochrome c oxidase subunit 8b (Cox8b), and long-chain acyl-CoA dehydrogenase (LCAD) in WT/Het and HSL-KO mice. mRNAs were determined by qRT-PCR in 6–7 individual mice. One-way ANOVA indicates a significant difference between genotypes at 3 days of treatment (***P < 0.001).

* and pharmacological inhibition of the lipase provides a direct test of its involvement in remodeling phenomena and additionally yields insights into the role that mobilized FFA might play (8, 17, 35).

As expected from previous work (7), a single injection of CL strongly upregulated the expression of chemotactic chemokines and led to the prominent infiltration of monocytes into the tissue. This effect was absent in HSL-null mice; however, these animals had persistent upregulation of inflammatory markers, which complicates interpretation (see below). To address this limitation, we examined the effects of acute pharmacological blockade of HSL in vivo and in vitro, using the selective inhibitor BAY (5). BAY alone had no effect on inflammatory gene expression but did significantly attenuate CL-induced proinflammatory signaling. The effects observed in vivo were reproduced in cultured adipocytes, strongly indicating that HSL-mediated mobilization of FFA triggers proinflammatory signaling within fat cells. In addition, it implicates the adipocyte as the source of inflammation during acute β3-AR activation in white adipose tissue.
The induction of mitochondrial biogenesis and expression of fatty acid oxidation genes by CL were significantly delayed in HSL-KO mice. We (25) previously reported that PPARα is required for the expansion of oxidative capacity in white adipose tissue during CL treatment. FFA are potent activating ligands for PPARα (18, 19), and FFA themselves can induce PPARα expression (33). It seems likely that reduced FFA release in HSL-KO animals contributes to the delay in adaptive metabolic remodeling. Nonetheless, HSL-KO mice still mobilize FFA, presumably through the action of other lipases, such as ATGL (10, 36), and this likely explains the residual metabolic remodeling that is apparent by 7 days of treatment in the animals. It has been recently shown that chronic administration of all-trans retinoic acid induces a brown adipose tissue-like phenotype in white adipose tissue (27). Presently, it is unclear whether CL treatment affects local generation of retinoic acid, which may have pro-oxidative effects. Regardless, CL-mediated production of retinoic acid would not explain the remodeling that occurs in HSL-KO mice after 7 days of CL treatment, since these mice would be expected to have reduced retinoic acid levels due the absence of retinyl esterase activity.

In summary, the present data indicate that HSL plays an important role in adipose tissue plasticity during acute and chronic activation of β3-AR. These data suggest that HSL-dependent generation of FFA initiates the proinflammatory effects of acute β3-AR activation and contributes to the adaptive upregulation of mitochondrial oxidative capacity during sustained stimulation. Nonetheless, the observation that acute and chronic inhibition of HSL can have pronounced, yet opposite, effects on inflammation suggests that the balance of HSL products and substrates is critical to the inflammatory status of fat tissue. Adipose tissue inflammation is thought to
affect systemic insulin sensitivity, and it will be an important goal of future work to identify the signaling mechanisms involved during β3-AR-induced inflammation.

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