Translocation of Hormone-Sensitive Lipase and Perilipin Upon Lipolytic Stimulation During the Lactation Cycle of the Rat

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The removal of the litter from lactating rats results in a decrease in the lipolytic response to catecholamines in maternal adipocytes; this effect can be prevented by concomitant treatment of the rats with growth hormone. The decrease in response to catecholamines following litter removal was not due to a change in the amount of either hormone-sensitive lipase (HSL) or perilipin per adipocyte or in the proportion of either of these proteins associated with the fat droplet. Incubation in vitro with isoproterenol did not cause any apparent net translocation of HSL to the fat droplet in adipocytes from the mature female rats in any state used in this study, but isoproterenol did cause a movement of perlipin away from the fat droplet. This translocation of perilipin was not altered by litter removal. Thus, the decrease in response to catecholamines found on litter removal from lactating rats appears to be due to a diminished ability to activate HSL associated with fat droplet.

LACTATION USUALLY RESULTS in the use of a substantial proportion of the lipid reserves of adipose tissue to help meet the demands for milk production and is associated with heightened response of lipolysis to catecholamines.[1] Lipid lost during lactation is then replaced on removal of the young or upon natural weaning, with reproductively active females undergoing cycles of lipid loss and accumulation.[2] Restoration of lipid reserves following litter removal is associated with an increase in lipogenesis[3-6] and a decrease in the lipolytic responsiveness of adipocytes to catecholamines.[7-10] The latter effect is prevented by treatment with growth hormone (GH) at time of litter removal.[8,9]

The rate-limiting step of lipolysis is catalyzed by hormone-sensitive lipase (HSL).[11-14] Stimulation of adipocytes with catecholamines elevates cyclic adenosine monophosphate (cAMP) levels and activates cAMP-dependent kinase (protein kinase A); protein kinase A serine phosphorylates and so activates HSL.[11-14] However, it is evident that lipolytic regulation is not due to HSL reversible phosphorylation alone, as there exists a discrepancy between the degree of activation of HSL in cell-free preparations (2- to 3-fold) and of the extraordinary stimulation of lipolysis observed in the intact adipocyte (50- to 100-fold) observed in some states.[11,14] This discrepancy is due, at least in part, to lipolytic stimulation resulting in both HSL activation and also translocation of HSL from the cytosol to the lipid droplet.[15,16] Additionally, recent evidence from HSL null mice suggests that another unidentified 'hormone-sensitive' triacylglycerol lipase(s), besides HSL, also participates in mediating lipolysis in adipocytes.[17] Activation of protein kinase A by catecholamines also results in the phosphorylation of perilipin, a protein found on the surface of the lipid droplet in adipocytes.[18-20] It was initially proposed that perilipin may directly anchor HSL to its substrate by acting as a 'docking' protein,[15] however, neither a yeast 2-hybrid screen[21] nor attempts to coimmunoprecipitate HSL and perilipin (unpublished data) have offered evidence of any direct interaction between these 2 proteins. Rather, evidence obtained from the expression of perilipin in differentiating cell lines suggests that perilipin may play a role in the packaging of lipid droplets.[22] Western blotting of adipocyte subcellular fractions has now shown that under certain conditions of lipolytic stimulation, perilipin-containing particles move away from the periphery of the large, central lipid droplet within freshly isolated[23] and 3T3-L1[24,25] adipocytes. Such characteristics have led to speculation that perilipins may have a role in the control of lipolysis, possibly acting as a barrier to HSL (and other putative triacylglycerol lipases) when in the nonphosphorylated state.[25]

The decrease in lipolytic response to catecholamines found on litter removal from lactating rats appears to be due to a defect in signal transduction downstream of protein kinase A activation.[9] In fact, the key impairment appears to be at the level of HSL interaction with the lipid droplet, as the HSL activity associated with the lipid droplet following a catecholamine challenge did not increase in adipocytes from litter-removed rats.[9] GH treatment of lactating rats at the time of litter removal prevented both the diminished lipolytic response to catecholamines and the loss of the ability of catecholamines to increase HSL activity of the fat droplet.[9] In this study, alterations in subcellular localization of both HSL and perilipin that occur upon lipolytic stimulation in lactating rats before and after litter removal and in response to GH treatment have been investigated to further elucidate the mechanism responsible for the diminished lipolytic response to catecholamines.

MATERIALS AND METHODS

Materials

Protease inhibitors, pepstatin, leupeptin, and antipain were from the Peptide Institute (Osaka, Japan). Collagenase was from Worthington Biochemical (Lakewood, NJ). Antiperilipin NH_2-terminal polyclonal antibody was raised in rabbits as described previously,[26] as was a

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polyclonal antibody raised against rat HSL/bacterial fusion protein.[27] Recombinant bovine growth hormone was supplied by Monsanto (St Louis, MO). All other reagents were from Sigma Chemical (Poole, Dorset, UK).

Animals

Female Wistar rats were raised in house at the Hannah Research Institute (Ayr, UK) where they were fed on irradiated CRM diet (Labsure, Poole, UK). If required, animals were mated at 2 to 3 months of age, and the number of pups per mother was adjusted to 10 within 24 hours after birth. If required, litters were removed, and relevant injections were begun on days 12 to 14 of lactation. Some rats, which had their litters removed, were injected with 1.5 mg of recombinant bovine growth hormone/injection, starting at the time of litter removal, whereas control virgin, lactating, and litter-removed rats were injected with carrier solution (0.1 mol/L $NaHCO_3$).[28] All injections were subcutaneous and were administered twice daily at 9:00 AM and 5:00 PM for 2 days; rats were not injected at 9:00 AM on day 3 and were killed by cervical dislocation at about 10:30 AM.

Adipocyte Isolation

Adipocytes were isolated by collagenase digestion of parametrial fat pads.[29] All manipulations were performed in Krebs-Ringer solution buffered with 25 mmol/L Hepes pH 7.4 (KRH) containing 2.5 mmol/L $CaCl_2$, 2.5 mmol/L $MgCl_2$, 3% bovine serum albumin (BSA), and 2 mmol/L glucose. A total of 200 nmol/L adenosine was included to suppress cAMP production and stimulation of cAMP-dependent protein kinase activity.[30] Following isolation, cells were shaken at 37°C for 1 hour. Cells were then washed in BSA-free buffer supplemented with 200 nmol/L adenosine. Aliquots of cells were removed into an equal volume of 2x sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole cell extracts.

Determination of Packed Cell and Mean Cell Volume

The packed cell volume (PCV) of the final suspension was determined by aspirating small aliquots into capillary hematocrit tubes and centrifugation in a microhematocrit centrifuge. For determination of adipocyte size, the diameter of 100 adipocytes was measured using a projection microscope (Projectina, Ayrshire, UK) at 145-fold magnification. The minimum cell diameter measured was 10 μm, and these values were used for calculation of the mean cell volume by the method of DiGirolamo et al.[31]

Preparation of Cytosolic and Fatcake Fractions for SDS-PAGE

Aliquots (300 μL) of adipocytes at approximately 20% PCV were incubated in 2 mL wells of 48-well tissue culture plates for 5 minutes at 37°C, either under 'basal' conditions, ie, supplemented with 200 nmol/L adenosine and 2 mmol/L glucose only, or in the presence of the additions mentioned in the text. KRH buffer (150 μL) was then removed from below the floating cells for assay of glycerol release, and the remaining cells lysed in 150 μL ice cold 50 mmol/L Tris-HCl buffer pH 7.4 containing 225 mmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L benzamidine, 1 μg/mL pepstatin, 1 μg/mL leupeptin and 1 μg/mL antipain, and 50 mmol/L NaF (buffer A). Following lysis, cells remained on ice for 15 minutes for the floating fatcake to solidify. The lysate was then vortexed vigorously and centrifuged at 13,000*g* at 4°C for 15 minutes. The cytosolic fraction was aspirated from below the solidified fatcake and 100 μL of cytosol added to an equal volume of 2x sample buffer for SDS-PAGE. The fatcake fraction was respun at 13,000*g* at 4°C for 15 minutes, and any contaminating cytosol aspirated and discarded. The fatcake was warmed to room temperature, 100 μL SDS sample buffer added, and the solution vortexed thoroughly. Following centrifugation at 13,000*g* at 4°C for 15 minutes, the fatcake protein extract was aspirated from below the floating fat layer for SDS-PAGE. Dilution factors and PCVs from each experiment were taken into consideration to ensure equivalent loading (per milliliter of packed cells) of the 2 fractions on subsequent SDS-PAGE.

SDS-PAGE and Western Blot Analysis

SDS-PAGE was performed using a Tris/glycine buffer system with Hoeffer mini-gel apparatus (10% gels).[32] For Western blotting, protein samples were transferred onto polyvinylidine difluoride (PVDF) membranes, probed with anti-HSL or antiperilipin antibodies, and the amount of immunoreactive protein (expressed as optical density) determined by Enhanced Chemiluminescence reagents from Amersham (Bucks, UK).

Estimation of Glycerol Release

Glycerol release was determined using a Sigma Diagnostics kit (cat. no. 337-B), which uses a procedure based on the original method of McGowan et al.[33] Aliquots (20 μL) were withdrawn from cell suspensions and added to 200 μL GPO-Trinder reagent and glycerol determined as described in the kit. Glycerol release is expressed as nmol glycerol/10^6 cells/min.

Statistical Analysis

Results were analyzed by analysis of variance (ANOVA); factors comprised physiologic state, isoproterenol, adenosine deaminase, and their interactions. Results are presented as means with either standard errors of differences (SED) from the ANOVA or SEMs.

RESULTS

Lipolysis

Adipocytes from lactating rats had a significantly ($P < .05$) smaller mean cell volume than adipocytes from virgin rats (Table 1). Litter removal from lactating rats resulted in an adipocyte mean cell volume intermediate between that of virgin and lactating rats; GH treatment of litter-removed rats resulted in mean cell volumes, which did not differ significantly from those of lactating rats (Table 1). Following incubation under basal conditions or with either 1 μmol/L isoproterenol, 1 U/mL adenosine deaminase or both, rates of lipolysis were measured by glycerol release. Lipolysis is expressed per 10^6 cells to take into consideration variations in cell volume (Fig 1). ANOVA (Table 2) demonstrated that there was a significant increase ($P < .001$) in the lipolytic rate upon stimulation of adipocytes with either isoproterenol or adenosine deaminase. There was also a significant ($P = .001$) interaction between isoproterenol

Table 1. Adipocyte Mean Cell Volume and HSL and Perilipin Content of Adipocytes From Rats in Different Physiologic States

	Mean Cell Volume (pL)	HSL (U/cell)	Perilipin (U/cell)
Virgin	333 ± 36(8)*	1.31 ± 0.23(7)	1.58 ± 0.18(8)
Lactating	221 ± 36(8)†	1.41 ± 0.22(8)	1.61 ± 0.18(8)
Litter-removed	258 ± 36(8)*†	1.45 ± 0.22(7)	1.84 ± 0.20(7)
Litter-removed, GH-treated	217 ± 36(8)*	1.50 ± 0.25(6)	1.62 ± 0.21(6)

NOTE. Values given are the mean ± SEM (from ANOVA) with the number of observations in parentheses. Values in a column without the same suffix, *†, differ $P < .05$.

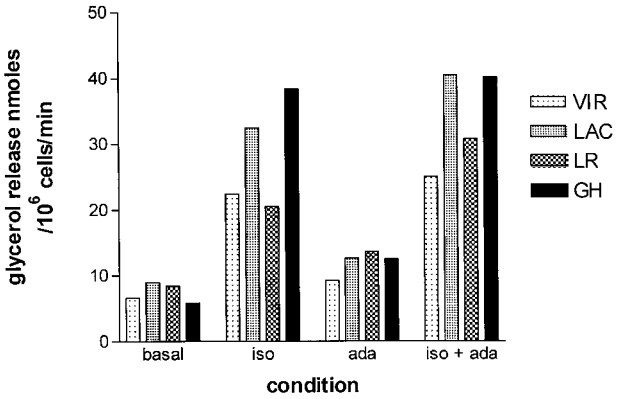

Fig 1. Glycerol release from rat adipocytes. Adipocytes were isolated from virgin (VIR), lactating (LAC), and litter-removed rats that had been treated for 2 days prior with (GH) or without (LR) growth hormone. Adipocytes were maintained under basal conditions or stimulated with 1 μmol/L isoproterenol (iso), 1 U/mL adenosine deaminase (ada), or both (iso+ada). Samples were removed for measurement of glycerol release. Results are means of 5 (VIR) or 8 (LAC, LR, GH) values. Results were analyzed by ANOVA (Table 2); SED for comparing effects of treatments of rats in the same physiologic state, 4.63 (VIR), 3.66 (LAC, LR, GH); SED for comparing effects of physiologic state, 6.63 (VIR with either LAC, LR, or GH), 5.24 (between LAC, LR, and GH).

and the physiologic state. Adipocytes from lactating rats were more lipolytically responsive to catecholamine than those from virgin animals ($P < .05$), and this heightened response was subsequently reversed ($P < .05$) upon litter removal; treatment with GH prevented this effect of litter removal. Adenosine deaminase increased ($P < .05$) glycerol release above that of basal conditions, albeit considerably less than that achieved by isoproterenol, and the effects of adenosine deaminase and isoproterenol were apparently additive. Response to adenosine deaminase did not vary with physiologic state (interaction nonsignificant, $P = .52$).

HSL/Perilipin Subcellular Localization and Translocation

Western blotting of whole cell extracts prepared from the parametrial adipocytes of virgin rats, lactating rats, and rats that had their litters removed at peak lactation treated with or without GH showed that there were no significant differences in the amount of HSL or perilipin per cell between any of the 4 groups of animals (Table 1).

Preparatory experiments showed that the adipocyte disruption procedure and the fractionation procedure resulted in essentially complete breakage of the adipocytes and minimal contamination of the fatcake fraction with cytosol from the fatcake fraction. Thus, Western blotting for protein kinase B[34] (a cytosol protein)[35] showed that this kinase was present in the cytosol fraction, but not in the fatcake fraction (less than 5% of amount in cytosol fraction, Fig 2, track C). Furthermore, SDS gel electrophoresis followed by staining for protein with Amido Black showed that several prominent protein bands present in the cytosol fraction were absent from the fatcake fraction (results not shown). Use of a hypotonic medium (10 mmol/L Tris instead of 220 mmol/L sucrose) for cell lysis also resulted in minimal contamination of the fatcake with cytosol (Fig 2, track D). By contrast, when adipocytes were disrupted at 22°C, substantial amounts of protein kinase B were found in the fatcake (Fig 2, tracks A and B), indicating either incomplete lysis of cells or contamination of the fatcake by cytosol. Separation of the fatcake and cytosol fractions by centrifugation also produced a pellet; this pellet was included in the cytosol fraction, but did not contain detectable HSL or perilipin (by Western blotting).

Separation of the fatcake and 13,000x*g* soluble fractions showed that incubation with isoproterenol in vitro had no effect on the proportion of HSL associated with the fatcake, and there

Table 2. ANOVA of Results of Figs 1 to 3

	Glycerol/Cell*				HSL†				Perilipin‡			
Source of Variation	DF	MS	VR	*P*	DF	MS	VR	*P*	DF	MS	VR	*P*
Rat stratum												
State	3	306	1.1	.37	3	1,229	1.3	.3	3	801	0.6	.62
Residual	23	278	5.2		25	949	12.8		23	1,338	6.7	
Rat units stratum												
Iso	1	12,008.0	224.8	<.001	1	2.5	0.03	.86	1	4,022.0	20.1	<.001
Ad	1	693.1	12.9	<.001	1	2,719.9	36.6	<.001	1	17.0	0.1	.77
Iso.ad	1	7.5	0.1	.71	1	162.5	2.2	.14	1	2.6	0.01	.91
State.iso	3	310.9	5.8	.001	3	94.5	1.3	.29	3	257.7	1.3	.28
State.ad	3	40.8	0.8	.52	3	76.4	1.0	.38	3	384.9	1.9	.13
State iso ad	3	49.7	0.9	.43	3	143.0	1.9	.13	3	81.9	0.4	.75
Residual	69	53.6			75	74.2			69	199.9		
Total	107				115				107			

NOTE. For ANOVA, fixed factors were iso (isoproterenol), ad (adenosine deaminase), and state and the random factor was rat (nested within state). GH treatment was considered as a 'state' for the purpose of this analysis.

Abbreviations: df, degrees of freedom; ms, mean sum of squares; vr, variance ratio.

*Glycerol released/10^6 cells/min (Fig 1).

†% HSL on fat (Fig 2).

‡% Perilipin on fat (Fig 3).

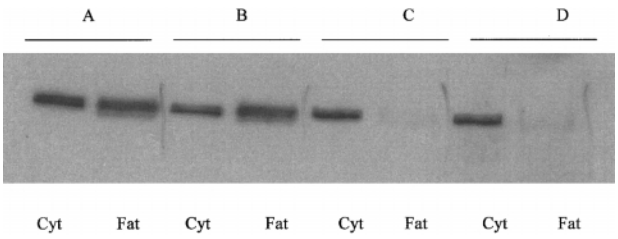

Fig 2. Lysis and fractionation of adipocytes. Adipocytes were lysed in either isotonic (225 mmol/L sucrose) (tracks A to C) or hypotonic (10 mmol/L Tris) (track D) buffer at either 22°C (tracks A and B) or 4°C (tracks C and D). Cells lysed at 4°C were kept on ice for 15 minutes prior to vortexing and centrifugation at 13,000*g*. Cells lysed at 22°C were either vortexed (track A) or not vortexed (track B) prior to centrifugation. Cytosol and fatcake fractions were subsequently subjected to SDS gel electrophoresis and blotted for protein kinase B. Cyt, cytosol; fat, fatcake.

was no effect of physiologic state or GH treatment on the proportion of HSL associated with the fatcake either under basal conditions or in the presence of isoproterenol (Table 2) (Fig 3). Unexpectedly, the addition of adenosine deaminase resulted in a decrease ($P < .001$, Table 2) in the proportion of HSL associated with the fatcake (25.9% and 16.2% of HSL associated with the fatcake in the absence and presence of adenosine deaminase, respectively, SED 1.6%, error degrees of freedom = 75); this effect was independent of the physiologic state and the presence or absence of isoproterenol.

Western blotting of infranatant and fatcake fractions with an antiperilipin antibody showed that incubation of adipocytes with isoproterenol caused a significant ($P < .001$) (Table 2, Fig 4) decrease in the proportion of perilipin associated with the fatcake (76.7% and 64.5%, respectively, in the absence and presence of isoproterenol, SED 2.72%, error degrees of freedom = 69); this effect was independent of the physiologic state. Addition of adenosine deaminase had no effect on the proportion of perilipin associated with the fatcake. The physiologic state of the rats had no significant effect on the proportion of perilipin associated with the fatcake (Table 2, Fig 4).

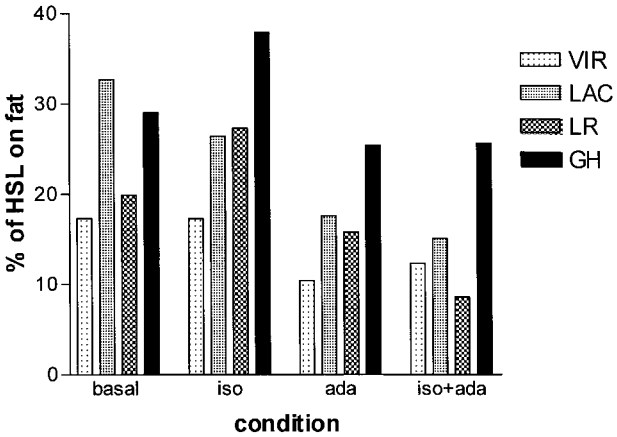

Fig 3. HSL translocation in adipocytes. Adipocytes were isolated from female Wistar rats and incubated as described in Fig 1. After 5 minutes of incubation, cells were lysed and infranatant and fatcake fractions prepared. Fractions were subjected to SDS-PAGE and Western blotted with anti-HSL antibodies. Densitometric analysis allowed a quantitative measurement of subcellular location of HSL. Results are means of 7 observations (8 for litter-removed rats) and were analyzed by ANOVA (Table 2). SED from ANOVA for comparing effects of treatments within a physiologic state, 4.78; SED for comparing effects of physiologic state, 11.75.

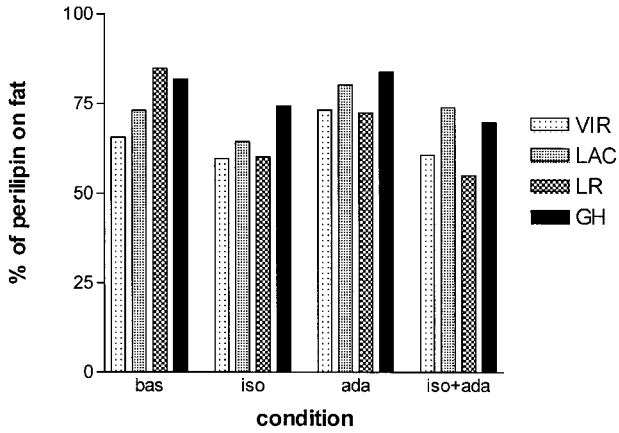

Fig 4. Perilipin translocation in adipocytes. Adipocytes were isolated from female Wistar rats and incubated as described in Fig 1. After 5 minutes of incubation, cells were lysed and infranatant and fatcake fractions prepared. Fractions were subjected to SDS-PAGE and Western blotted with antiperilipin antibodies. Densitometric analysis allowed a quantitative measurement of subcellular location of perilipin. Results are means of 7 observations; (6 for GH-treated rats) and were analyzed by ANOVA (Table 2). SED from ANOVA for comparing effects of treatments within a physiologic state, 7.87; SED for comparing effects of physiologic state, 12.25.

DISCUSSION

The fractionation procedure used to investigate possible changes in HSL or perlipin translocation between the cytosol and the fatcake is identical to that used previously.[23] In the earlier study,[23] it was found that isoproternol induced a substantial translocation of HSL from the cytosol to the fatcake in adipocytes from young male rats, but had no effect on HSL translocation in mature male rats. This study also showed that translocation of HSL was very rapid and complete within 4 minutes of the addition of isoproterenol. Glycerol release was monitored to ensure that litter removal and treatment with GH altered response to isoproterenol as found previously.[8,9] As glycerol was assayed in samples of incubation medium (that is intracellular glycerol was not measured), it is possible that the actual rate of lipolysis was underestimated due to incomplete equilibrium of glycerol between cell and medium in the short incubation period, but this approach had the advantage of assessing both glycerol release and determining HSL and perilipin translocation in the same population of cells. Indeed, as found previously,[8,9] the removal of litters from lactating rats resulted in a decrease in the lipolytic response to catecholamines, and this effect was prevented by injection of GH. However, an increase in lipolytic response to catecholamines in lactating rats over that of virgin animals was found in the

present study, which is contrary to earlier studies using longer incubations (25 minutes or more) in which no difference between lacatating and virgin animals was observed.[7,9,36]

It was shown previously that activation of protein kinase A by isoproterenol was not altered by litter removal, indicating that the decrease in lipolytic response was downstream of this key kinase.[9] Furthermore, it was shown that the decrease in lipolytic response upon litter removal was not associated with any change in total HSL activity under basal or stimulated conditions, but with a failure of catecholamine to increase HSL activity associated with the fatcake.[9] This led to speculation that some alteration in the ability of HSL to translocate to the lipid droplet was involved during the lactation/litter removal transition, which was prevented by GH.[9] However, the data presented here suggests that no significant translocation of HSL protein from the cytosol to the fat droplet is occurring during acute lipolytic stimulation in any of the 4 states investigated. Similarly, a previous study with mature male rats failed to find any translocation of HSL to the fat droplet in response to isoproterenol stimulation.[23] By contrast, isoproterenol caused a significant stimulation of HSL translocation to the fat droplet in adipocytes from young male rats (body weight, less than 220 g)[15,23] and also in 3T3 L1 adipocytes.[16] In young male rats, there was a significant correlation between the rate of isoproterenol-stimulated lipolysis and the proportion of HSL associated with the fatcake.[23] Interestingly, isoproterenol induced much greater rates of lipolysis in adipocytes from young male rats than in adipocytes from mature male rats.[23] This suggests that high rates of lipolysis require translocation of HSL to the fat droplet, whereas lower rates of lipolysis can be achieved without any such translocation. The relatively low rates of lipolysis found in the mature female rats used in the present study, which are similar to those found previously with mature male rats,[23] are consistent with this idea.

The mature female rats used in the present study also resemble the mature male rats used previously,[23] in that isoproterenol induced a loss of some perilipin from the fat droplet to the infranatant fraction. However, there appear to be quantitative differences, as 90% of perilipin was associated with the fat droplet in the basal state in mature male rats, and this decreased to 45% on stimulation with isoproterenol.[23] The relevance of this movement of perilipin from the fat droplet in response to catecholamines to the stimulation of lipolysis in mature female and male rats is unknown. However, it is consistent with the suggestion that perilipin located on the surface of the large, central fat droplet of the adipocyte acts as a barrier to HSL (and other putative triacylglycerol lipases) in the basal state.[25,37] Curiously, isoproterenol caused no movement of perilipin from the fat droplet in adipocytes from young male rats.[23]

While the behavior of the lipolytic system of mature female rats appears very similar to that of mature male rats,[23] female rats differ from male rats in that even at about 6 weeks of age, isoproterenol did not induce a significant translocation of HSL to the fat droplet. In adipocytes from young female rats 13.7% $\pm$ 5.3% and 23.3% $\pm$ 1.8% of HSL was associated with the fatcake before and after stimulation of adipocytes with 1 μmol/L isoproterenol. Furthermore, and in keeping with the lack of translocation of HSL, the rate of isoproterenol-stimulated lipolysis in adipocytes from 6-week-old female rat adipocytes was only 55.0 $\pm$ 11.7 nmol glycerol release/min/mL packed cells, compared with 173.3 $\pm$ 18.4 nmol glycerol released/min/mL packed cells in male rats of a similar age[23]; values for female rats are mean $\pm$ SEM of 4 observations. These various observations support the view that high rates of lipolysis, as found in young male rats, require translocation of HSL to the fat droplet, whereas lower rates of lipolysis, as found in female rats and in mature male rats, do not involve net translocation of HSL and appear to involve activation of HSL already associated with the fat droplet. The observations also raise the possibility that the reason for HSL translocation in young male rats is to avoid relatively high rates of basal lipolysis in the unstimulated state, which might be anticipated if a large proportion of HSL remained permanently associated with the fat droplet. One other factor, which cannot be assessed at present, is the contribution of the novel triacylglycerol lipase(s) indicated by studies with null HSL mice.[17] The intracellular localization of the putative lipase is not known, and currently it is not possible to distinguish the activity of the novel lipase from that of HSL, so its contribution to lipolysis in rats of different age, sex, and physiologic state cannot be determined.

The finding that adenosine deaminase decreased the proportion of HSL associated with the fatcake, but increased the rate of lipolysis, was unexpected and appears paradoxical. The implication is that the HSL remaining with the fat droplet must be more active in cells treated with adenosine deaminase, but the molecular basis of this is unclear. Alternatively other 'hormone-sensitive' lipases may be involved. As the effect was observed in the presence and absence of isoproterenol, changes in protein kinase A activity is unlikely to be the explanation. That variations in HSL activity on the fat droplet can occur through mechanisms, in addition to phosphorylation by protein kinase A, is also indicated by results for litter removal. Litter removal from lactating rats has no effect on the ability of catecholamines to activate protein kinase A, but results in a loss of the ability to activate HSL associated with the fat droplet.[9] The recent report that HSL as a dimer has markedly greater activity against triacylglycerol than HSL existing as a monomer[38] provides a potential explanation for this observation. In any case, the present study shows that the diminished lipolytic response to catecholamines found after litter removal from lactating rats is not due to a change in translocation of either HSL to, or perilipin from, the fat droplet, rather it would appear that the diminished lipolytic effect of isoproterenol found on litter removal appears to be due to an impairment in the ability to activate HSL already associated with the fat droplet (or to activate unidentified triacylglycerol lipases); the molecular basis of this remains to be elucidated.

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