Optimized conditions for measuring lipolysis in murine primary adipocytes

Srikant Viswanadha and Constantine Londos[1]

Laboratory of Cellular and Developmental Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-8028

Abstract **The current literature on lipolysis in murine primary adipocytes is rife with experiments performed under conditions not optimized for reproducible and reliable results. Here, we present conditions for optimizing the measurement of lipolysis in murine adipocytes. We demonstrate that adenosine management is of paramount importance in evaluating the lipolytic response under basal and stimulated conditions. Also, adipocyte concentrations in the 10,000–15,000 cells per milliliter range produce a greater increase in stimulated lipolysis than higher concentrations, and the response is further enhanced by agitating the cells.**—Viswanadha, S., and C. Londos. **Optimized conditions for measuring lipolysis in murine primary adipocytes.** ***J. Lipid Res.*** **2006.** 47: **1859–1864.**

Supplementary key words glycerol • adenosine • cell concentrations

Nearly 20 years ago, we embarked on a series of experiments to explore the basis for the high variability among published studies on lipolysis in isolated adipocytes. At that time, the vast majority of such studies were performed with rat adipocytes, isolated according to the classical method of Rodbell (1). We published several papers on the handling and manipulation of isolated adipocytes (2–4) required to provide optimal and reproducible results in the measurement of lipolysis, because most published studies revealed a high level of variability in results. In recent years, an increasing number of papers have appeared on the behavior of isolated murine adipocytes, and once again, the literature reveals a high level of variability in the magnitude of stimulation achieved with β-adrenergic receptor agonists or with other stimulants that increase cAMP concentrations and PKA activity.

In our earlier studies, several factors emerged as important for the reproducible assay of adipocyte lipolysis, not the least of which was careful control of the ambient adenosine concentration (4). Adipocytes contain a high-affinity A1 adenosine receptor that is linked to G_i, the adenylyl cyclase inhibitory G protein. For two reasons, it is important to control the adenosine concentration. First, in the absence of adenosine, adipocytes may exhibit constitutively high lipolytic activity. Second, based on various factors, such as the season of the year and immediate dietary history, basal activity may be relatively high, thus blunting the magnitude of stimulation over basal that may be achieved with a lipolytic stimulant (i.e., signal-to-noise ratio).

Another important feature is the control of fatty acids that are released upon stimulation of lipolysis. Fatty acids are strongly inhibitory toward the receptor-mediated activation of adenylyl cyclase (5). Appropriately low fatty acid concentrations are achieved using low adipocyte concentrations, such that the ambient BSA in the assay medium is sufficient to bind all free fatty acids. Likewise, adequate mixing of the incubation medium is also essential to provide access of free fatty acids to the medium BSA. Otherwise, the adipocytes rise to the top of the incubation tube and present excess fatty acids to adjacent adipocytes in the upper layer, thus inhibiting adenylyl cyclase activity. Here, we present conditions for obtaining reproducible and optimal results for measuring lipolysis in primary murine adipocytes.

MATERIALS AND METHODS

Reagents

All reagents were obtained from Sigma-Aldrich (St. Louis, MO) and were prepared and manipulated in an Adipocyte Incubation Solution (AIS) unless indicated otherwise. The AIS contained Krebs Ringer Bicarbonate HEPES buffer (containing 10 mM bicarbonate and 30 mM HEPES, pH 7.4) supplemented with 3% (w/v) fatty acid free bovine albumin fraction V (number 820025; ICN Biomedical, Inc.). Where indicated, the adenosine deaminase-resistant (ADA) A1 receptor agonist, (−)-N^6-(2-phenylisopropyl)-adenosine (PIA), was present at 100 nM, (−)-isoproterenol (ISO) was present at 10 μM, and ADA was present at 1 U/ml. It is important to use enzyme stock supplied in ammonium sulfate and not glycerol suspensions, because the glycerol content in-

Manuscript received 7 February 2006 and in revised form 23 March 2006 and in re-revised form 18 April 2006.

Published, JLR Papers in Press, May 4, 2006.
DOI 10.1194/jlr.D600005-JLR200

[1] To whom correspondence should be addressed.
e-mail: deanl@intra.niddk.nih.gov

terferes with the glycerol assay used in these studies. The cells were isolated in AIS fortified with 500 nM adenosine and 3 mg/ml type 1 collagenase (Worthington Biomedical Corp., Lakewood, NJ). The optimal concentration of adenosine used for adipocyte isolation and washes was based on the finding that mouse adipocytes were ~10-fold less sensitive compared with rat adipocytes (J. Tansey and C. Londos, unpublished observation). Because of lot-to-lot variation in collagenase activity, the optimal concentration of collagenase may range from 1 to 3 mg/ml; we determined that 3 mg/ml collagenase stock was optimal for the digestion of adipose tissue samples in this study.

Isolation of adipocytes

Procedures involving animals were carried out in accordance with the guidelines specified by the National Institute of Diabetes and Digestive and Kidney Diseases Animal Care and Use Committee. Adipocytes were isolated according to the classical Rodbell method (1) with modifications, such as the inclusion of adenosine in the medium during cell isolation to suppress lipolysis, as recommended by Honnor, Dhillon, and Londos (3, 4). Briefly, three male C57BL/6J mice (8–10 weeks old) (Taconic, Rockville, MD) were anesthetized using Forane® (Baxter, Deerfield, IL) and euthanized by cervical dislocation. Inguinal and epididymal fat pads (~2 g) were harvested and placed in weighing boats containing PBS (pH 7.4) at room temperature. Fat pads were blotted dry, weighed, and minced thoroughly (~2–3 mm pieces in diameter) in collagenase solution (3 ml/g of adipose). This mixture was transferred to a 30 ml narrow-mouthed polypropylene bottle (Nalgene, Rochester, NY) and incubated at 37°C with shaking at 220 rpm for 1 h. After digestion, the mixture was filtered through a 250 μm gauze mesh into a 50 ml conical polypropylene tube (Becton Dickinson, Franklin Lakes, NJ) and allowed to stand for 2–3 min. The infranatant containing the collagenase solution was carefully removed using a long needle and syringe. The floating layer of adipocytes was washed three times with 10 ml of the adenosine-replete AIS. The adipocyte solution was centrifuged for 30 s at 800 rpm, and the infranatant was removed and discarded. Typically, 25 (125,000 cells), 50 (250,000 cells), 100 (500,000 cells), 250 (1,125,000 cells), or 500 (2,250,000 cells) μl of packed cells was resuspended in 5 ml of AIS for subsequent distribution into assay tubes. All data were normalized to cell number. Cell counts were obtained by calculation after determining average cell sizes and TAG content according to Fine and Di Girolamo (6).

Lipolysis

Basal and stimulated lipolysis were determined by assaying glycerol released after cell incubation at 37°C for 1 h. After brief agitation of the suspension to obtain a homogeneous mixture of adipocytes, 200 μl was added to 5 ml polypropylene 12 × 75 mm incubation tubes containing 400 μl of AIS plus other lipolytic agents (PIA, ADA, ISO) and incubated for 1 h at 37°C. Two hundred microliters of the adipocyte suspension was also added to tubes containing AIS without lipolytic reagents and incubated at room temperature for 1–2 min to establish starting glycerol values. After incubation, 200 μl of infranatant was pipetted into microfuge tubes and stored at −20°C until assayed for glycerol.

Glycerol assay

The glycerol assay used in these studies was the highly sensitive assay reported by Bradley and Kaslow (7) as adapted to 96-well plates by Brasaemle et al. (8). Briefly, 1 μCi of [γ-^{32}P]ATP and 20 μl of glycerokinase (Roche, Indianapolis, IN) were added to 5 ml of stock assay buffer (2 mM $MgCl_2$, 100 nM triethanolamine-HCl, 2 mg/ml BSA, and 120 μM ATP). Fifty microliters of known standards and glycerol samples in duplicate were mixed with 50 μl of the above assay buffer in each well of a 96-well plate and incubated at 37°C for 30 min. Next, 100 μl of acid mix (2 N $HClO_4$ + 0.2 mM H_3PO_4) was added to the wells, and the plate was again incubated at 90°C for 30 min. After cooling to room temperature, 50 μl of 100 mM ammonium molybdate and 50 μl of 200 mM triethanolamine were added to the wells. The plate was centrifuged at 1,000 *g* for 20 min. One hundred microliters of the supernatant was removed, and radioactivity was determined with a liquid scintillation analyzer (Packard, Downers Grove, IL). Glycerol concentrations were determined based on the standard curve and expressed as nanomoles per 10^6 adipocytes.

Statistical analysis

Data are reported as means ± SD. Data were analyzed using ANOVA, and *P* values were determined using SAS® software (SAS Institute, Inc., Cary, NC). Post hoc tests were performed using Bonferroni's multiple comparisons when the overall treatment effects were found to be significant. Differences were deemed significant at $P < 0.05$.

RESULTS AND DISCUSSION

A comparison of recently published studies on the lipolysis of isolated primary murine adipose cells reveals a wide range of responsiveness to lipolytic stimulation (**Table 1**). Although several publications report stimulations of 30-fold or greater, the majority show much more modest stimulations of <10-fold, and some as little as 3-fold or less. It is difficult to evaluate such literature because many papers do not reveal the conditions under which basal activity was measured, and most do not provide cell concentration values. The data below will address the manipulations that may be used to enhance the magnitude of stimulation, either by reducing the basal value or by amplifying the stimulated values. The important variables to consider are *1*) cell concentration, *2*) agitation or shaking of the incubation mixture, and *3*) composition of the incubation medium, especially management of the ambient adenosine.

The importance of adenosine management is illustrated in **Fig. 1**, which reveals glycerol release in the presence or absence of PIA, ADA, or both in 10K cells under basal conditions when the tubes were incubated without shaking (Fig. 1A) or with shaking at 150 rpm (Fig. 1B). Removal of PIA or PIA and ADA from the cell incubation medium provoked a significant increase in basal lipolysis compared with tubes in which only ADA was removed or both PIA and ADA were added. Removal of ADA from the incubation mixture did not affect the stimulated lipolysis. Glycerol release was ~2- to 3-fold higher with shaking at 150 rpm compared with tubes that were incubated without shaking. Note especially the high basal release of glycerol in the presence of ADA only, which converts adenosine to inosine, which is not recognized by adenosine receptors. There are two sources of adenosine to consider under our conditions. First, we add exogenous adenosine to suppress lipolysis during the course of cell isolation and washing; second, adenosine will be present secondary to cell lysis leading to leakage of adenylyl nucleotides, which are

TABLE 1. Conditions for lipolysis from selected studies in chronological order

First Author / Year	Additions to the Incubation Medium		Cells Assayed[a]	Lipolytic Stimulation[b]
	Basal Conditions	Stimulated Conditions		
Reaven / 1988 (21)	None	10^{-6} M ISO (60 min)	10^5 cells/ml	~12-fold
Tozzo / 1997 (22)	1 U/ml ADA, 10 μM PIA	1 U/ml ADA, 10 μM PIA, 100 μM ISO (15 min)	100 μl (20,000 cells)	~10-fold
Coe / 1999 (23)	None	100 μM ISO (30 min)	100 μl of adipocytes	2- to 3-fold
Shaughnessy / 2000 (24)	4 μg/ml ADA, 100 nm/l PIA	4 μg/ml ADA, 100 nmol/l PIA, 10^{-6} mol/l norepinephrine		25- to 42-fold
Martinez-Botas / 2000 (25)	1 U/ml ADA	1 U/ml ADA, 2 μM CL316,243 (60 min)	0.350×10^6 cells/ml	3-fold
Wang / 2001 (26)	1 U/ml ADA, 10 μM PIA	1 U/ml ADA, 10 μM PIA, 10 μM CL316,243 (60 min)	100 μl of adipocytes	6.15-fold
Tansey / 2001 (27)	1 U/ml ADA, 100 nM PIA	1 U/ml ADA, 100 nM PIA, 10 μM ISO (60 min)		~30-fold
Hertzel / 2002 (28)	1 U/ml ADA, 10 μM PIA	1 U/ml ADA, 10 μM PIA, 100 μM ISO (30 min)	25,000 cells	3-fold
Takahashi / 2002 (29)	None	10^{-6} M epinephrine	20,000–40,000 cells	10-fold
Lucas / 2003 (30)	1 U/ml ADA, 100 nmol/l PIA	1 U/ml ADA, 100 nmol/l PIA, 100 μM ISO (90 min)		9-fold
Sztalryd / 2003 (20)	1 U/ml ADA, 100 nM PIA	1 U/ml ADA, 10 μM ISO (60 min)		~40-fold
Fortier / 2004 (31)	5 μg/ml ADA, 10 μM PIA	5 μg/ml ADA, 10 μM PIA, 10^{-5} M ISO (120 min)	3,000 cells/50 μl	13.14-fold
Cohen / 2004 (32)	1 U/ml ADA, 100 nmol/l PIA	1 U/ml ADA, 100 nmol/l PIA, 5 μmol/l CL316,243 (60 min)		30-fold
Iglesias-Osma / 2004 (33)	None	10 μM forskolin (90 min)		4.8-fold

All incubations for lipolysis were performed at 37°C. ADA, adenosine deaminase; ISO, isoproterenol; PIA, (−)-N^6-(2-phenyl-isopropyl)-adenosine.
[a] Number of cells used for lipolysis if indicated in publication.
[b] Ratio of stimulated-to-basal lipolysis.

hydrolyzed to adenosine. Note that in the absence of any adenosine receptor agonist (−PIA, +ADA), basal activity may be extraordinarily high, blunting the ratio of stimulated-to-basal activity. Such data replicate the findings we reported with rat adipocytes under what we termed the "ligand-free" condition. On the other hand, the addition of PIA, the adenosine receptor agonist, or exclusion of ADA exerts a powerful adenosine receptor-mediated inhibition of basal activity, and stimulation with ISO under these adenosine replete conditions (−ADA) increases to nearly 40- to 50-fold. It is also evident that both basal and stimulated activities are enhanced upon shaking during incubation of the cells. It should be noted that with the identical cell preparation, one may easily elicit a wide range of ISO-mediated stimulations, from ~2-fold up to nearly 50-fold, by merely adjusting incubation conditions. In contrast to our findings, the addition of ADA to the incubation medium did not alter basal lipolysis in human adipocytes (9, 10). These discrepancies may be attributed to the very low concentration of adipocytes in the human cell incubation mixture (<5,000 vs. 10,000 cells per incubation) or to species differences (human vs. mouse).

In our earlier studies on rat adipocytes, we did not highlight the importance of using appropriate cell concentrations during the measurement of lipolytic rates (3, 4). The importance of cell concentration and agitation (shaking) are illustrated in **Fig. 2A, B**. Under both conditions (i.e., with or without shaking), the magnitude of stimulation of glycerol release by ISO over basal conditions decreased dramatically with increasing cell concentrations. Moreover, shaking the tubes at 150 rpm increased stimulated lipolysis by ~3-fold in the 5K and 10K samples compared with tubes that were not agitated during incubation. We had shown previously that without shaking there is a rapid increase in cAMP shortly after the application of ISO, followed by a steep decline (11). This phenomenon was known as "peaking," which resulted from fatty acid-mediated inhibition of cAMP formation. Edens, Leibel, and Hirsch (12) demonstrated a reduction in fatty acid re-esterification in shaken adipocyte incubations compared with unshaken incubations. Increased shaking speed provides for greater mixing of the fatty acids with the medium BSA, which binds and neutralizes the fatty acids. With more rapid shaking, the peaking disappears and activation of PKA reaches a steady state that does not decline with time (3, 11).

Optimal shaking speed is a function of the geometry of the incubation vessel and the volume of the incubation medium. The shaking speed should be such that the adipocytes are distributed uniformly throughout the medium and do not rise to the surface and become concentrated near the top of the tube. On the other hand, shaking should not be so vigorous that cells are damaged. Optimal shaking speed may be determined by visual inspection of the incubation tube: the mixture should present as a uniform milky solution without a visible upper layer of concentrated adipocytes. Thus, the shaking speed can be reduced when a larger tube or flask is used for incubation.

Even at optimal shaking speeds, the negative effects of high cell concentrations are not overcome (Fig. 2B). High cell concentrations compounded with higher medium FFA concentrations reduced reesterification and tended to decrease the rate of lipolysis in human adipocytes (12). Similarly, increasing adipocyte concentration from 0.168 $\times 10^6$ to 1.252 $\times 10^6$ cells/ml reduced the conversion of glucose to glyceride-glycerol but increased the production

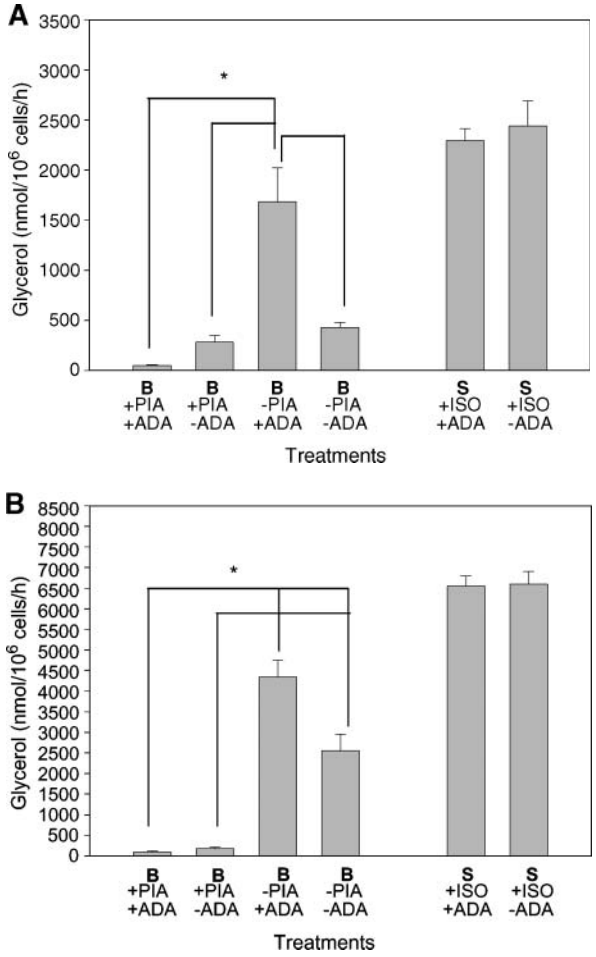

Fig. 1. Glycerol release in isolated adipocytes (10,000 cells per incubation tube) incubated under varying basal (B) or stimulated (S) conditions. Basal lipolysis was determined in the presence or absence of (−)-N^6-(2-phenyl-isopropyl)-adenosine (PIA), adenosine deaminase (ADA), or both, whereas stimulated lipolysis was determined in the presence of isoproterenol (ISO) with or without ADA. Suspensions of adipocytes were incubated at 37°C without shaking (A) or with shaking at 150 rpm (B). Values represent averages ± SD of triplicate incubations (from a single adipocyte isolation procedure). SD bars not visible were smaller than the thickness of the line describing the data bar. * Significant differences between treatments joined directly by lines ($P < 0.05$).

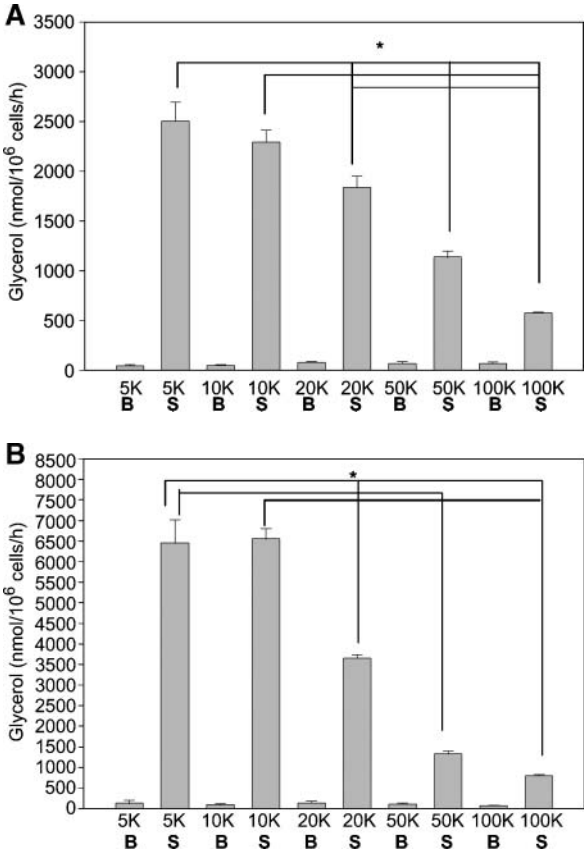

Fig. 2. Change in basal (B) and stimulated (S) lipolysis using different adipocyte concentrations. Suspensions of adipocytes were incubated at 37°C without shaking (A) or with shaking at 150 rpm (B). Values on the vertical axis represent glycerol produced (± SD) of triplicate incubations (from a single adipocyte isolation procedure). SD bars not visible were smaller than the thickness of the line describing the data bar. * Significant differences between treatments (stimulated lipolysis) joined directly by lines ($P < 0.05$). The overall treatment effect was also significant for basal lipolysis ($P < 0.05$). Basal activities were measured with ADA plus PIA and stimulated with ISO plus ADA. The values under the bars indicate the total number of cells per incubation tube × 10^3; thus, 10K = 10,000 cells per 600 μl = ∼16,000 cells/ml.

of glyceride-fatty acids (13). In the current experiment, the increase from 10,000 to 20,000 cells per milliliter crossed from the optimal to the deleterious range of cell concentration. In general, optimal results were obtained with ∼10,000 to 15,000 cells per milliliter in the final incubation medium. This range is far too low to accurately measure glycerol levels by the standard spectrophotometric procedures. Accordingly, we now routinely use the radiometric assay method, which measures glycerol accurately in the very low nanomole range. Alternatively, bioluminescence (9) and fluorometric (14) methods can also be used to measure glycerol release from adipocyte lipolysis.

Data normalization among adipocyte preparations is a critical factor in interpreting results. It is imperative that data be normalized according to cell number. Normalization to protein concentration is not advisable, because the vast majority of protein in a typical adipocyte suspension is the BSA added to protect the cells, whereas the protein contributed by the cells is but a minuscule fraction of the total protein present. Similarly, measurement of DNA values may be misleading, because much of the DNA present may be contributed by smaller cells that cling to the adipocytes (15). Typically, estimation of cell numbers using DNA values results in a 5- to10-fold overestimation. (Unfortunately, the problem with using DNA measurements to obtain cell numbers is not well documented in the current literature. We were alerted to this discrepancy by Dr. Susan K. Fried and confirmed that DNA measurements led to a considerable overestimation of cell numbers over the values obtained by calculating cell number using size measurement and lipid content, as described herein.) Thus, the most efficacious way to

normalize lipolysis data is to obtain a cell count, as described by Fine and Di Girolamo (6).

It should be noted that the 50-fold stimulations observed in this study were obtained with relatively young mice on a standard chow diet with adipocytes modest in size (~60 μm in diameter). Basal lipolysis, however, is increased in obesity (16). We also found reduced ISO-mediated stimulation (10- to 15-fold) with adipocytes from mice fed a high-fat diet compared with animals fed a chow diet (unpublished data).

It should also be understood that the basal activities, as well as the magnitude of stimulation achieved under the experimental conditions described here, may not reflect those activities in vivo. These conditions are designed to permit comparisons of adipocyte behavior in different mouse models as measured by different investigators. The so-called basal activities measured in the present studies are most likely artificially low and are designed to permit viewing of maximal stimulated activities.

Hormone-sensitive lipase had for many years been considered the major, if not the only, lipase responsive during the course of lipolytic stimulation (17). More recently, the demonstration that adipocyte triglyceride lipase may contribute significantly to this reaction has introduced another variable that must be considered in the lipolytic reaction (18). Also, any analysis of the protein kinase A-dependent lipolytic reaction must consider the role of perilipin A. In any careful dissection of the lipolytic reaction, especially upon introduction of various siRNAs, it is essential to determine the different contributions to the lipolytic reaction by different lipases and perilipin (19, 20). Such analyses, therefore, will require a rigid and reproducible method for measuring both basal and hormone-stimulated lipolysis.

This project was supported by the National Institute of Diabetes and Digestive and Kidney Diseases/National Institutes of Health intramural research program.

REFERENCES

1. Rodbell, M. 1964. Adipocyte isolation. *J. Biol. Chem.* **239:** 375–380.
2. Londos, C., R. C. Honnor, and G. S. Dhillon. 1985. cAMP-dependent protein kinase and lipolysis in rat adipocytes. III. Multiple modes of insulin regulation of lipolysis and regulation of insulin responses by adenylate cyclase regulators. *J. Biol. Chem.* **260:** 15139–15145.
3. Honnor, R. C., G. S. Dhillon, and C. Londos. 1985. cAMP-dependent protein kinase and lipolysis in rat adipocytes. II. Definition of steady-state relationship with lipolytic and antilipolytic modulators. *J. Biol. Chem.* **260:** 15130–15138.
4. Honnor, R. C., G. S. Dhillon, and C. Londos. 1985. cAMP-dependent protein kinase and lipolysis in rat adipocytes. I. Cell preparation, manipulation, and predictability in behavior. *J. Biol. Chem.* **260:** 15122–15129.
5. Fain, J. N., and R. E. Shephard. 1976. Inhibition of adenosine 3′:k′-monophosphate accumulation white fat acids, lactate, and beta-hydroxybutyrate. *J. Lipid Res.* **17:** 377–385.
6. Fine, J. B., and M. Di Girolamo. 1997. A simple method to predict cellular density in adipocyte metabolic incubations. *Int. J. Obes. Relat. Metab. Disord.* **21:** 764–768.
7. Bradley, D. C., and H. R. Kaslow. 1989. Radiometric assays for glycerol, glucose and glycogen. *Anal. Biochem.* **180:** 11–16.
8. Brasaemle, D. L., D. M. Levin, D. C. Adler-Wailes, and C. Londos. 1999. The lipolytic stimulation of 3T3-L1 adipocytes promotes the translocation of cytosolic hormone-sensitive lipase to the surfaces of lipid storage droplets. *Biochim. Biophys. Acta.* **1483:** 251–262.
9. Mauriege, P., P. Imbeault, D. Prud'homme, A. Tremblay, A. Nadeau, and J. P. Despres. 2000. Subcutaneous adipose tissue metabolism at menopause: importance of body fatness and regional fat distribution. *J. Clin. Endocrinol. Metab.* **85:** 2446–2454.
10. Arner, P., and P. Engfeldt. 1987. Fasting-mediated alteration studies in insulin action on lipolysis and lipogenesis in obese women. *Am. J. Physiol.* **253:** E193–E201.
11. Kuroda, M., R. C. Honnor, S. W. Cushman, C. Londos, and I. A. Simpson. 1987. Regulation of insulin-stimulated glucose transport in the isolated rat adipocyte. cAMP-independent effects of lipolytic and antilipolytic agents. *J. Biol. Chem.* **262:** 245–253.
12. Edens, N. K., R. L. Leibel, and J. Hirsch. 1990. Mechanism of free fatty acid re-esterification in human adipocytes in vitro. *J. Lipid Res.* **31:** 1423–1431.
13. Digirolamo, M., S. V. Thacker, and S. K. Fried. 1993. Effects of cell density on in vitro glucose metabolism by isolated adipocytes. *Am. J. Physiol.* **264:** E361–E366.
14. You, T., D. M. Berman, A. S. Ryan, and B. J. Nicklas. 2006. Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. *J. Clin. Endocrinol. Metab.* **89:** 1739–1746.
15. Klyde, B. J., and J. Hirsh. 1979. Isotopic labeling of DNA in rat adipose tissue: evidence for proliferating cells associated with mature adipocytes. *J. Lipid Res.* **20:** 691–704.
16. Fried, S. K., R. L. Leibel, N. K. Edens, and J. G. Kral. 1993. Lipolysis in intraabdominal adipose tissue of obese women and men. *Obes. Res.* **1:** 443–448.
17. Holm, C., T. Osterlund, H. Laurell, and J. A. Contreras. 2000. Molecular mechanisms regulating hormone-sensitive lipase and lipolysis. *Annu. Rev. Nutr.* **20:** 365–393.
18. Zimmermann, R., J. G. Strauss, G. Haemmerle, G. Schoiswohl, R. Birner-Gruenberger, M. Riederer, A. Lass, G. Neuberger, F. Eisenhaber, A. Hermetter, et al. 2004. Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science.* **306:** 1383–1386.
19. Tansey, J. T., C. Sztalryd, E. M. Hlavin, A. R. Kimmel, and C. Londos. 2004. The central role of perilipin A in lipid metabolism and adipocyte lipolysis. *IUBMB Life.* **56:** 379–385.
20. Sztalryd, C., G. Xu, H. Dorward, J. T. Tansey, J. A. Contreras, A. R. Kimmel, and C. Londos. 2003. Perilipin A is essential for the translocation of hormone-sensitive lipase during lipolytic activation. *J. Cell Biol.* **161:** 1093–1103.
21. Reaven, G. M., H. Chang, and B. B. Hoffman. 1988. Additive hypoglycemic effects of drugs that modify free-fatty acid metabolism by different mechanisms in rats with streptozocin-induced diabetes. *Diabetes.* **37:** 28–32.
22. Tozzo, E., L. Gnudi, and B. B. Kahn. 1997. Amelioration of insulin resistance in streptozotocin diabetic mice by transgenic overexpression of GLUT4 driven by an adipose-specific promoter. *Endocrinology.* **138:** 1604–1611.
23. Coe, N. R., M. A. Simpson, and D. A. Bernlohr. 1999. Targeted disruption of the adipocyte lipin-binding protein (ap2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J. Lipid Res.* **40:** 967–972.
24. Shaughnessy, S., E. R. Smith, S. Kodukula, J. Storch, and S. K. Fried. 2000. Adipocyte metabolism in adipocyte fatty acid binding protein knockout mice ($aP2^{-/-}$) after short-term high-fat feeding: functional compensation by the keratinocyte fatty acid binding protein. *Diabetes.* **49:** 904–911.
25. Martinez-Botas, J., J. B. Anderson, D. Tessier, A. Lapillone, B. H. J. Chang, M. J. Quast, D. Forenstein, K. H. Chen, and L. Chan. 2000. Absence of perilipin results in leanness and reverses obesity in $Lepr^{db/db}$ mice. *Nat. Genet.* **26:** 474–479.
26. Wang, S. P., N. Laurin, J. Himms-Hagen, M. A. Rudnicki, E. Levy, M. F. Robert, L. Pan, L. Oligny, and G. A. Mitchell. 2001. The adipose tissue phenotype of hormone-sensitive lipase deficiency in mice. *Obes. Res.* **9:** 119–128.
27. Tansey, J. T., C. Sztalryd, J. Gruia-Gray, D. L. Roush, J. V. Zee, O. Gavrilova, M. L. Reitman, C. X. Deng, C. Li, A. R. Kimmel, et al. 2001. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc. Natl. Acad. Sci. USA.* **98:** 6494–6499.

28. Hertzel, A. V., A. Bennaars-Eiden, and D. A. Bernlohr. 2002. Increased lipolysis in transgenic animals overexpressing the epithelial fatty acid binding protein in adipose cells. *J. Lipid Res.* **43:** 2105–2111.
29. Takahashi, S., and N. Satozawa. 2002. The 20-kD human growth hormone reduces body fat by increasing lipolysis and decreasing lipoprotein lipase activity. *Horm. Res.* **58:** 157–164.
30. Lucas, S., G. Tavernier, C. Tiraby, A. Mairal, and D. Langin. 2003. Expression of human hormone-sensitive lipase in white adipose issue of transgenic mice increases lipase activity but does not enhance in vitro lipolysis. *J. Lipid Res.* **44:** 154–163.
31. Fortier, M., S. P. Wang, P. Mauriege, M. Semache, L. Mfuma, H. Li, E. Levy, D. Richard, and G. A. Mitchell. 2004. Hormone-sensitive lipase-independent adipocyte lipolysis during beta-adrenergic stimulation, fasting, and dietary fat loading. *Am. J. Physiol. Endocrinol. Metab.* **287:** E282–E288.
32. Cohen, A. W., B. Razani, W. Schubert, T. M. Williams, X. B. Wang, P. Iyengar, D. L. Brasaemle, P. E. Scherer, and M. P. Lisanti. 2004. Role of caveolin-1 in the modulation of lipolysis and lipid droplet formation. *Diabetes.* **53:** 1261–1270.
33. Iglesias-Osma, M. C., M. J. Garcia-Barrado, V. Visentin, M. F. Pastor-Mansilla, S. Bour, D. Prevot, P. Valet, J. Moratinos, and C. Carpene. 2004. Benzylamine exhibits insulin-like effects on glucose disposal, glucose transport, and fat cell lipolysis in rabbits and diabetic mice. *J. Pharmacol. Exp. Ther.* **309:** 1020–1028.