Impaired Fat Storage Capacity in Adipocyte Precursors of I versus C57BL Mice (42956)

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Abstract. **I mouse strain displays adipocyte hypoplasia responsible for smaller fat pad size compared with C57BL mice. We investigated possible alterations in the proliferation and/or differentiation capacity of preadipocytes from the stroma-vascular fraction of adipose tissue in the I mouse strain. Control C57BL and I mice were studied at 8 weeks of age, and both adipose and stromal cells were isolated from epididymal and inguinal adipose tissue localizations.**

Results showed that the lower epididymal adipose mass in I mice was accompanied by a decrease in stromal cell number compared with C57BL mice. In inguinal fat pads, total cell number in the stroma-vascular fraction was unmodified; lipoprotein lipase activity significantly increased in stromal cells from I mice compared with control mice. In this depot, further characterization of cells from the stroma-vascular fraction by separation of cells according to density showed an increased number of preadipocytes in the I mouse whole stromal cell population. These preadipocytes seemed unable to undergo terminal maturation, thus leading to a decrease in the number of mature adipocytes.

These results indicated that resistance to fat accumulation in I mice is characterized by site-dependent impairment of both the proliferative rate and the differentiation capacity of adipocyte precursors. [P.S.E.B.M. 1989, Vol 192]

I strain mice characteristically gain less weight and have a lower body fat content and smaller amounts of adipose tissue than other nonobese mouse strains such as C57BL (1, 2). Moreover, I strain mice are resistant to fat accumulation with age (2) and high-fat diets (3). In line with this unusually low ability to accumulate fat, a reduction in food efficency and high oxygen consumption has been reported in I mice (4) along with greater *in vivo* glucose tolerance than other mouse strains (5). Recently, increased insulin-mediated glucose metabolism has been observed in fat cells (6), but not in isolated diaphragm (7) from I mice, suggesting that adipose tissue may be the site responsible for altered lipid storage capacity. Further studies have emphasized this observation by demonstrating that I mice exhibit both higher lipolysis and fatty acid synthesis as well as smaller adipocytes than control mice (8). In I mice, it has been shown that the reduction in epididymal fat pad size resulted from both adipose cell hypotrophy and hypoplasia (8). This hypoplasia contributed to the aggravation of metabolic alterations exhibited by isolated adipocytes. The objective of this study was thus to determine whether such a decrease in fat cell number is a common feature of adipose tissue development in I mice. Two adipose sites which develop at different periods of postnatal life in rodents were investigated.

It is now well established that mature fat cells derived from proliferation and differentiation of adipocyte precursors in the stroma-vascular fraction (i.e., non-lipid-filled cells) of adipose tissue (9, 10). During the last several years, *in vitro* culture of either established (11) preadipose cell lines or (12) preadipocytes isolated from adipose tissue has demonstrated that differentiation is characterized by the induction of enzymes of the triacylglycerol pathway, leading to the accumulation of fat droplets in cells (11–13). Among these, lipoprotein lipase, which emerges very early during adipose conversion (13, 14), and glycerol-3-phosphate dehydrogenase, which is induced just prior to lipid accumulation in cells (15, 16), are now considered as suitable markers of the differentiation process. In the stroma-vascular fraction of adipose tissue, preadipocytes at different steps of adipose conversion can be isolated and numbered using a density gradient separation procedure, providing valuable information on

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the capacity of the tissue to form new adipocytes under various metabolic conditions (17). This technique has enabled us to establish that the increased differentiation capacity of preadipocytes is partly responsible for overdevelopment of adipose tissue in nutritionally induced obesity in the newborn rat (18).

In this study, we wished to determine whether the decreased number of mature fat cells in adipose tissue from I mice, resistant to fat accumulation, could be related to impairment of the proliferation rate and/or differentiation capacity of preadipocytes from the stroma-vascular compartment.

Materials and Methods

Animals. Male I/Crgl and C57BL/6 Crgl mouse strains were obtained from the Cancer Research Laboratory (University of California, Berkeley, CA) or from first generation litters of parents from the Cancer Research Laboratory Center at San Diego State University. Mice were maintained at room temperature (23°C) with a 12-hr alternate light and dark cycle and had free access to water and pelleted Wayne Lab-Blox F6 (Continental Grain Co., Chicago, IL). Studies were performed when the mice were 8 weeks of age. Male mice only were used.

Preparation of Isolated Stromal Cells. Mice were killed between 9 AM and 10 AM by cervical dislocation. For each experiment, total epididymal or inguinal adipose tissues was removed, weighed, and pooled (four to seven mice of each strain) and cut into pieces prior to collagenase treatment. Tissues were digested by collagenase treatment according to Rodbell (19) for 1 hr at 37°C in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 3% bovine serum albumin (Fraction V; Sigma Chemical Co., St. Louis, MO), 5 m*M* glucose, and 1 mg/ml collagenase (Boehringer, Mannheim, West Germany). After incubation, the undigested tissue pieces were removed by filtration through a 190-μm nylon mesh. Mature fat cells were allowed to float to the surface and the infranatant (i.e., stroma-vascular cell fraction) was aspirated. Floating cells were washed twice with collagenase-free buffer, and the infranatant and washes were centrifuged at 360*g* for 5 min. The pellet consisting of stromal cells was resuspended in appropriate buffer, filtered through a 25-μm nylon mesh, and counted using a Coulter Counter Channelizer ZBI-C1000 system equipped with a 100 μm in diameter orifice tube. Under our setting for counting conditions, we ensured that red blood cells were not taken into account.

Mature Adipose Cell Number Determination. Fat cell size was measured on aliquots of fat cell suspension by using a photometric method as described previously (6). From these determinations, mature fat cell number was calculated by dividing the tissue lipid content by fat cell size. Tissue lipid content generally ranged from 52 to 78% of wet weight. The mice depots contained 518 and 678 mg of lipid/g for inguinal tissue and 680 and 780 mg of lipid/g for epididymal tissue in I and control mice, respectively.

Determination of Lipoprotein Lipase and G3PDH Activities. Lipoprotein lipase (LPL) activity was determined in stromal cells from epididymal or inguinal adipose tissue according to the method described by Nilsson-Ehle and Schotz (20). After pelleting, the stromal cells were resuspended in ice-cold NH_4OH-NH_4Cl buffer (50 m*M*, pH 8.1) containing heparin (4 IU/ml), sonicated, and assayed directly for LPL activity. Glycerol-3-phosphate dehydrogenase (G3PDH) activity was assayed on stromal cell cytosolic supernatants obtained by centrifugation (1 hr at 39,000*g*) using the spectrophotometric procedure described by Kozak and Jensen (21) and modified by Wise and Green (15).

Separation of Stromal Cells by Density Gradient Centrifugation. The total stroma-vascular cell population was counted and further separated by density gradient centrifugation using a 36% Percoll solution (Pharmacia, Uppsala, Sweden) as described previously (17). Briefly, a continuous density gradient (from 1.018 to 1.075 g/liter) was performed by centrifugation at 10,000*g* for 30 min. The cells (5×10^6/ml) were layered on top of the gradient and centrifuged at 1000*g* for 25 min at room temperature: four density fractions were collected from the top of the tube after controlling the density ranges of each fraction with density marker beads (Pharmacia). The densities of the fractions ran from 1.018 to 1.033 (Fraction I), from 1.033 to 1.049 (Fraction II), from 1.049 to 1.062 (Fraction III), and from 1.062 to 1.075 (Fraction IV).

Statistical Analysis. All values are expressed as the mean ± SE. Statistical differences between mouse strains or between adipose tissue sites within the genotype were determined by Student's *t* test.

Results

At 8 weeks of age, body weight was significantly lower in I mice than in the C57BL mice. Inguinal and gonadal fat pads were generally smaller in I than in C57BL mice, and site to site variations in fat pad weight differed in the two strains: in control mice, the epididymal fat pads were heavier than the inguinal fat pads, whereas in I mice, they were lighter.

Marked differences between the two strains were observed in the number of mature fat cells (Table I). In I mice, the number of fat cells was substantially lower (inguinal, 32%; epididymal, 49%) than the respective cell number from C57BL mice. In addition, adipose cell size was similar in both strains in inguinal localization: data previously reported (6) were 33 ± 3 and 37 ± 3 ng of lipid/cell in I and C57BL mice, respectively. In epididymal tissue of I mice, both the number and size of mature fat cells were reduced, adipocytes weighing 60% less ($P < 0.05$) than those of controls (54 ± 10 and 89 ± 10 ng of lipid/cell). Table I also shows marked

differences between the two strains in the stromal cell number of the two different adipose depots studied. In C57BL mice, the number of cells in the stroma-vascular fraction of inguinal tissue was about twice the epididymal tissue value in spite of a lower tissue mass at this localization. Thus, when expressed per gram of adipose tissue, the contribution of cells from the stroma-vascular compartment to fat pad mass was significantly different in the two adipose localizations: $4.31 \pm 0.28 \times 10^6$/g vs $14.26 \pm 2.19 \times 10^6$/g for epididymal and inguinal tissue, respectively. No significant difference between the two strains was observed in total stromal cell number in the inguinal depot. In contrast, the stromal cell number in epididymal pads was markedly reduced in I mice compared with C57BL mice (−59%).

Table II shows LPL and G3PDH activities measured in isolated stromal cells from either epididymal or inguinal adipose tissue in I and in C57BL mice, and taken as differentiation markers of the capacity of these cells to undergo adipose conversion. When expressed per milligram of cell protein, there were no site to site variations in G3PDH and LPL activities except in control mice, in which gonadal tissue exhibited significantly higher LPL activity than inguinal tissue. In epididymal tissue, when expressed per milligram of protein or per whole stromal cell compartment, there was no difference between the two strains of mice in either LPL or G3PDH activities. In contrast, in inguinal tissue, LPL activity per whole stromal cell compartment was significantly increased in I mice compared with C57BL mice. In this tissue, no difference between the two groups of mice was detectable for G3PDH activity.

Since stromal cells isolated from the inguinal adipose tissue of I mice exhibited higher LPL activity than that of cells from control mice, we also characterized the adipocyte precursor pool in the stroma-vascular fraction. The whole stromal cell population isolated from inguinal adipose tissue was further fractionated according to the density of cells, in order to quantitatively assess the number of preadipocytes in the two strains of mice (Table III). In the two strains, 60% of cells were recovered in the three lightest fractions and can thus be considered as preadipocytes at different levels of differentiation. However, in Fraction I, there were 90% more cells in I mice when compared with controls; this is in agreement with higher levels of differentiation marker enzyme activity (LPL) observed in the whole stromal compartment of these mice. Taken

Table I. Adipocyte and Stromal Cell Number in Epididymal and Inguinal Adipose Tissues from 8-Week-Old I Strain and C57BL Mice[a]

	I	C57BL	*P*
Body weight (g)	21.3 ± 0.3	22.8 ± 0.3	<0.001
Epididymal adipose tissue			
2-pad weight (mg)	127 ± 9*	309 ± 6**	<0.001
Stromal cell number ($\times 10^6$)	0.79 ± 0.10**	1.33 ± 0.11*	<0.01
Fat cell number ($\times 10^6$)	1.37 ± 0.24*	2.69 ± 0.05**	<0.001
Inguinal adipose tissue			
2 pad weight (mg)	146 ± 15	211 ± 8	<0.01
Stromal cell number ($\times 10^6$)	2.81 ± 0.36	3.02 ± 0.49	NS
Fat cell number ($\times 10^6$)	2.41 ± 0.39	3.52 ± 0.14	<0.001

[a] The total number of mice studied was 41 for the I strain and 40 for C57BL. The values are mean ± SE from six to seven experiments which represented a pool of adipose tissues from 4 to 11 mice each. $P > 0.05$ are considered as not significant (NS). Statistical difference between epididymal and inguinal tissues within a strain is indicated by * $P < 0.05$, ** $P < 0.001$.

Table II. Lipoprotein Lipase and Glycerol-3-Phosphate Dehydrogenase Activities in Stromal Cells Isolated from Epididymal and Inguinal Tissues of I and C57BL Mice

	Epididymal adipose tissue		Inguinal adipose tissue	
	I	C57BL	I	C57BL
Lipoprotein lipase[a]				
Protein (milliunits/mg)	185.6 ± 26.2[b]	169.4 ± 17.3	129.5 ± 21.8	77.2 ± 14**
Whole stromal cell compartment (milliunits)	34.1 ± 5.6	46.4 ± 6.3	57.1 ± 6.6*	37.1 ± 6.0
Glycerol-3-phosphate dehydrogenase[c]				
Protein (milliunits/mg)	4.90 ± 0.69	4.32 ± 1.02	4.09 ± 0.92	3.16 ± 0.90
Whole stromal cell compartment (milliunits)	0.79 ± 0.14	0.99 ± 0.20	2.34 ± 0.49**	1.37 ± 0.2

[a] One enzyme unit represents 1 μmol of free fatty acid hydrolyzed/hr.
[b] Values are mean ± SE. Statistical differences between mouse strains are indicated by * $P < 0.05$; differences between epididymal and inguinal tissue within the mice strain are indicated by ** $P < 0.01$.
[c] One enzyme unit represents 1 μmol of NADH oxidized/min.

Table III. Distribution of Stromal Cells from Inguinal Adipose Tissue According to Density[a]

	Cell number/tissue ($\times 10^6$)		
	I	C57BL	*P*
Fraction I $1.018 < d < 1.033$	0.685 ± 0.126	0.358 ± 0.044	<0.01
Fraction II $1.033 < d < 1.049$	0.451 ± 0.133	0.367 ± 0.097	NS
Fraction III $1.049 < d < 1.062$	0.696 ± 0.120	0.611 ± 0.205	NS
Fraction IV $1.062 < d < 1.075$	1.123 ± 0.077	1.356 ± 0.270	NS

[a] Values are mean ± SE of five experiments. $P > 0.05$ are considered as not significant (NS). Cell recovery after density gradient was 91 ± 5% and 98 ± 3% for control and I mice, respectively.

together, these results showed that the inguinal adipose tissue of I mice was characterized by more numerous preadipocytes, exhibiting greater capacity to undergo the adipocyte differentiation process.

Discussion

The aim of this study was to delineate possible alterations in the stroma-vascular compartment of adipose tissue which might be related to the unusually low fat accretion in the I strain. To examine this question, two different adipose localizations were studied, thus providing information on the cellular development of adipose tissue in terms of site specificity.

Results in the C57BL strain reveal considerable variations between adipose localizations in terms of the number of nonadipose cells in tissue: the stromal cell number in inguinal adipose tissue was 2-fold higher than at the epididymal site, in spite of reduced tissue mass at this localization. Our findings suggest that site to site differences may be found in both *in vivo* replication and differentiation of adipose precursor cells. In agreement with this hypothesis, a previous *in vitro* study demonstrated a lower proliferative rate of preadipocytes from epididymal adipose tissue than from other tissues (22). The differentiation capacity of preadipocytes in the stroma-vascular compartment of adipose tissue, evaluated by measurement of activity of early differentiation marker enzymes such as LPL, appeared to be elevated in epididymal adipose tissue compared with the inguinal depot. This higher potential for undergoing adipose conversion, along with the low proliferative rate of preadipocytes found in the stromal cells of the epididymal fat pad, could provide a possible explanation for both the enlarged size and reduced number of mature adipocytes in epididymal compared with inguinal tissue.

In I mice as compared with control mice, our results show that the number of mature fat cells is markedly reduced in both inguinal and epididymal tissue, indicating that adipocyte hypoplasia in I mice seems to occur as an early feature in adipose tissue development. This result contrasts with an earlier report (8) showing that significant hypoplasia of epididymal tissue in I mice did not emerge before 16 weeks of age. In that study, fat cell size values in 8- to 9-week-old mice were higher than those obtained in this study. This discrepancy between the two studies is mainly due to the use of different methods for fat cell size and number determinations.

Some alterations in either *in vivo* proliferation or differentiation of adipocyte precursors were detected in each depot studied. In epididymal adipose tissue, the ability to produce adipocytes from precursors, evaluated by the activity of differentiation marker enzymes, was unaffected in 8-week-old I mice compared with control mice. Nevertheless, in these animals, the total number of cells in the stroma-vascular compartment was markedly reduced, suggesting low cell proliferation capacity in this compartment. Obviously, this defect is responsible for the aggravation of mature adipocyte hypoplasia exhibited by I mice during adulthood, since most DNA synthesis in the epididymal fatpad occurred before sexual maturity (23). In inguinal tissue, excessive LPL activity in stromal cells of I mice suggests that these cells exhibit an increased capacity to differentiate. In order to further confirm this possible alteration in I mice, we compared the distribution of stromal cells from the two strains of mice according to their density. A separation procedure based on cell triglyceride content was first described for isolating a homogeneous population of preadipocytes from newborn rats that are able to extensively differentiate in primary culture (24). It enabled us to show that, in 10-day-old rats, the differentiation capacity in primary culture of cells from the whole stromal compartment could be related to the number of cells distributing in the lightest density fractions (17). This procedure could be used to quantitatively assess the *in vivo* proportion of differentiating cells in the stroma vascular fraction (18). A recent study has established that stromal cells from mature rodents retain the capacity to differentiate *in vitro* similar to that of very young animals when they are maintained in appropriate serum-free medium (25). These findings argue for the appropriateness of this method for providing valuable information on the capacity to form new adipocytes in adult rodents. Our data, showing a 90% increase in cell number in the lightest fraction in I mice, in combination with excessice LPL activity in the stromal compartment, strongly suggest that the adipocyte precursors of I mice have a greater capacity to differentiate than those of control mice.

Adipose conversion, extensively investigated in culture systems, is a sequential process which involves the induction of marker enzymes. Among these, the emergence of LPL takes place in the very early steps of adipose conversion in cells which are still devoid of lipids (16). Further terminal differentiation of cells, which requires massive cell lipid filling, is related to the induction of G3PDH activity (15).

In this study, cells which were recovered in the lightest density fraction had begun to accumulate lipids; it is thus likely that LPL was expressed in these cells. Accordingly, in I mice, the increased number of cells in the lightest density fraction could account for the higher LPL activity observed in the whole stromal cell compartment. Nevertheless, in these stromal cells, since G3PDH activity remained low and there was a limited amount of triglycerides, terminal maturation had not yet begun. Consequently, this excess of cells in the early stages of adipose conversion, along with mature adipose cell hypoplasia, led us to speculate that the translocation of preadipocytes into the mature fat cell compartment was impaired. Indeed, our results show that the induction of LPL activity was insufficient to allow terminal maturation of preadipocytes, suggesting that *in vivo* adipose conversion could be modulated at different step of the ongoing process. Such a blockage in the differentiation process has been observed in a totally different pathology, i.e., obesity in which young obese Zucker rats displayed adipocyte hypoplasia with a high level of LPL activity in preadipocytes (26). Recently, it has been shown that retinoic acid treatment of 3T3-F442A cells *in vitro* could mimic such a situation, since the emergency of LPL activity was not followed by an increase in G3PDH activity and terminal lipid filling of cells (27).

Resistance to fat accumulation exhibited by I mice, in addition to causing metabolic changes in the mature fat cell compartment, is characterized by impairment of the differentiation capacity of adipocyte precursors, leading to the lower capacity of I mice to produce adipocytes. Why these alterations are expressed in terms of either proliferation or differentiation of the precursors, depending on the tissue localization, remains to be elucidated. Differences in the developmental stages of epididymal and inguinal tissue (the first to develop during postnatal life) could be partly responsible for this phenomenon.

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1. Fenton PF, Carr CJ. The nutrition of the mouse. X. Studies on the utilization of high and moderately low protein diets for growth in four strains of mice. J Nutr **43**:441–450, 1951.
2. Fenton PF, Dowlings MT. Studies on obesity. I. Nutritional obesity in mice. J Nutr **49**:319–331, 1953.
3. Fenton PF, Carr CJ. The nutrition of the mouse. XI. Response of four strains to diets differing in fat content. J Nutr **45**:225–233, 1951.
4. Hoover-Plow JL, Nelson B. Oxygen consumption in mice (I strain) after feeding. J Nutr **115**:303–310, 1985.
5. Lyon JB. Difference in glucose utilization among inbred strains of mice. Fed Proc **16**:215, 1956.
6. Hoover-Plow JL, Guerre-Millo M, Lavau M. Increased insulin-mediated glucose metabolism in fat cells from I versus C57BL mice. Proc Soc Exp Biol Med **184**:410–415, 1987.
7. Hoover-Plow JL, Gross GR, Mayer SE. Muscle glucose utilization in mice (I strain) resistant to obesity. Fed Proc **39**:726, 1979.
8. Hoover-Plow JL. Adipose tissue development and metabolism in two strains of mice. Nutr Rep Int **29**:1171–1179, 1984.
9. Pilgrim CH. DNA synthesis and differentiation in developing white adipose tissue. Dev Biol **26**:69–76, 1972.
10. Björntorp P, Karlsson M, Peztorft H, Pettersson P, Sjöstrom L, Smith U. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. J Lipid Res **19**:316–324, 1978.
11. Green H. The adipose conversion of 3T3 cells. In: Ahmad A, Russel TR, Schultz J, Werner R. Eds. 10th Miami Winter Symposium on Differentiation and Development. New York: Academic Press, pp13–36, 1978.
12. Bjorntorp P, Karlsson H, Petterson P, Sypniewska G. Differentiation and function of rat adipocyte precursor cells in primary culture. J Lipid Res **21**:714–723, 1980.
13. Gaben-Cogneville AM, Quignard-Boulangé A, Aron Y, Brigant L, Jahchan R, Pello JY, Swierczewski E. Development under the control of insulin of lipogenic enzymes, lipoprotein lipase, isoproterenol and glucagon sensitivity in differentiating rat preadipocytes in primary culture. Biochim Biophys Acta **805**:212–260, 1984.
14. Wise LS, Green H. Studies of lipoprotein lipase during the adipose conversion of 3T3 cells. Cell **13**:233–242, 1978.
15. Wise LS, Green H. Participation of one isoenzyme of cytosolic glycerophosphate deshydrogenase in the adipose conversion of 3T3 cells. J Biol Chem **254**:273–275, 1979.
16. Vannier C, Gaillard D, Grimaldi P, Amri E, Djian P, Cermolacce C, Forest C, Etienne J, Negrel R, Ailhaud G. Adipose conversion of ob 17 cells and hormone-related events. Int J Obes **9**(suppl 1):41–53, 1985.
17. Dugail I, Quignard-Boulangé A, Ardouin B, Brigant L. A method for separating cultured preadipocytes according to their density: Application to stromal cells from overfed suckling rats. In Vitro **22**:375–380, 1986.
18. Dugail I, Quignard-Boulangé A, Dupuy F. Role of adipocyte precursors in the onset of obesity induced by overfeeding in suckling rats. J Nutr **116**:524–535, 1986.
19. Rodbell M. Metabolism of isolated fat cells. I. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem **239**:375–380, 1964.
20. Nilsson-Ehle P, Schotz MC. A stable radioactive substrate emulsion for assay of lipoprotein lipase. J Lipid Res **17**:536–541, 1976.
21. Kozak LP, Jensen JT. Genetic and developmental control of multiple forms of L-glycerol 3-phosphate dehydrogenase. J Biol Chem **249**:7775–7781, 1974.
22. Djian P, Roncari DAK, Hollenberg CH. Influence of anatomic site and age on the replication and differentiation of rat adipocyte precursors in culture. J Clin Invest **72**:1200–1208, 1983.
23. Greenwood MRC, Hirsch J. Postnatal development of adipocyte cellularity in the normal rat. J Lipid Res **15**:474–483, 1974.
24. Gaben-Cogneville AM, Aron Y, Idriss G, Jahchan T, Pello JY, Swierczewski E. Differentiation under the control of insulin of rat preadipocytes in primary culture. Isolation of homogeneous cellular fraction by gradient centrifugation. Biochim Biophys Acta **762**:437–444, 1983.
25. Deslex S, Negrel R, Ailhaud G. Development of a chemically defined serum-free medium for differentiation of rat adipocyte precursor cells. Exp Cell Res **168**:15–30, 1987.
26. Quignard-Boulangé A, Dugail I, Ardouin B, Brigant L, Dupuy F, de Gasquet P. Impaired adipocyte differentiation in vivo in the Zucker rat. Int J Obes **7**:378, 1983.
27. Pairault J, Quignard-Boulangé A, Dugail I, Lasnier F. Differential effects of retinoic acid upon early and late events in adipose conversion of 3T3 preadipocytes. Exp Cell Res **177**:27–36, 1988.