Modulation of lipid-synthesizing enzymes by insulin in differentiated ob17 adipose-like cells

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ob17 cells convert into adipose-like cells when maintained in the presence of physiological concentrations of insulin and tri-iodothyronine. After this conversion, insulin removal from differentiated ob17 cells gives within 24–48 h a large decrease in fatty acid synthetase, glycerol 3-phosphate dehydrogenase and acid:CoA ligase activities, as well as in the rate of fatty acid synthesis determined by [14 C]acetate incorporation into lipids. All parameters are restored by insulin addition to initial values within 24–48 h. Dose–response curves of insulin on the restoration of glycerol 3-phosphate dehydrogenase activity and of fatty acid synthesis give half-maximally effective concentrations close to 1 nM, in agreement with the affinity for insulin of the insulin receptors previously characterized in these cells. Immunotitration experiments indicate that the changes in the specific activity of fatty acid synthetase are due to parallel changes in the cellular enzyme content. Therefore the ob17 cell line should be a useful model to study the long-term effects of insulin on the modulation of lipid synthesis in adipose cells.

Adipose tissue is exceeded only by the liver in importance for lipogenesis in rodents (Volpe & Vagelos, 1976). Previously, extensive work on fat tissue *in vivo* has implicated insulin as the major hormone regulating, on a long-term basis, endogenous fatty acid synthesis through the action of fatty acid synthetase (Saggerson & Greenbaum, 1970; Bruckdorfer *et al.*, 1972; Volpe & Vagelos, 1974); insulin also appears to regulate the entry of exogenous fatty acids through the action of lipoprotein lipase (Cryer *et al.*, 1976; Cryer, 1981). The adaptive changes induced by insulin in fatty acid synthetase content of fat tissue have been well established *in vivo*, but a direct stimulatory effect of insulin remains to be shown, whereas the adaptive changes brought about by this hormone in lipoprotein lipase content of fat tissue have been shown *in vivo* (Jansen *et al.*, 1978) and more recently *in vitro* (Vannier *et al.*, 1982). In the latter study, preadipocyte ob17 cells (Négrel *et al.*, 1978) were used to show, for the first time, that conversion into adipocytes is accompanied by a net increase in cell content of lipoprotein lipase, and that this increase is modulated by physiological concentrations of insulin

and tri-iodothyronine. The present paper describes a study of the direct effect of insulin on fatty acid synthetase content in differentiated ob17 cells, and also the effect of insulin on the activity of glycerol 3-phosphate dehydrogenase, a key enzyme for providing the glycerol 3-phosphate required for triacylglycerol synthesis.

Experimental

Cell culture

ob17 cells were plated at 2×10^3 – 3×10^3 cells/cm² in 35 mm-diameter dishes in the presence of Dulbecco's Modified Eagle's Medium supplemented with 10% (v/v) foetal-calf serum, 200 units of penicillin/ml, 50 μ g of streptomycin/ml, 33 μ M-biotin and 17 μ M-pantothenate. This medium is termed 'standard medium'. Unless otherwise stated, confluent cells (approx. 4 days after seeding) were maintained in standard medium supplemented with 17 nM-insulin and 1.5 nM-tri-iodothyronine for 12–15 days. Media were changed every 2 days. This fortnight period after confluence has been shown previously to be sufficient for maximal adipose conversion (Gharbi-Chihi *et al.*, 1981). This con-

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version corresponds to the completion of post-confluent mitoses of cells susceptible to such conversion (Djian *et al.*, 1982) and to the attainment of maximal specific activity for fatty acid synthetase and glycerol 3-phosphate dehydrogenase (Gharbi-Chihi *et al.*, 1981). Control cells were maintained after confluence in standard medium supplemented with 1.5 nM-tri-iodothyronine, but in the absence of insulin. Cell numbers were determined with a Coulter counter. When indicated, insulin removal was performed by rinsing the cells three times at 37°C (20 min each) with Dulbecco's Modified Eagle's Medium containing 10% foetal-calf serum and 1.5 nM-tri-iodothyronine in an atmosphere of air/CO₂ (19:1).

Cell-free extracts and enzyme assays

At 18–24 h after the last change of medium, cells were rinsed twice at 37°C with phosphate-buffered saline, pH 7.4 (140 mM-NaCl, 3 mM-KCl, 8 mM-Na₂HPO₄, 1.5 mM-KH₂PO₄). Cell homogenates (from at least two pooled dishes) were obtained by homogenization in a Potter–Elvehjem homogenizer (20 strokes) with 20 mM-Tris/HCl buffer, pH 7.5; they were used directly for assays. A supernatant fraction (15 min at 10000 g and 2°C) was used for the immunotitration experiments. Fatty acid synthetase activity was determined in cell homogenates to which were added 300 mM-potassium phosphate, pH 7.0, 5 mM-EDTA and 5 mM-dithiothreitol. Fatty acid synthetase, acid:CoA ligase, glycerol 3-phosphate dehydrogenase and lactate dehydrogenase were assayed as previously described (Gharbi-Chihi *et al.*, 1981). All enzyme assays were performed at least in duplicate. Enzyme activities are expressed in munits/mg of protein, i.e. in nmol of product formed/min per mg of protein, except for fatty acid synthetase, expressed in nmol of [¹⁴C]malonyl-CoA incorporated into fatty acids/min per mg of protein. Inter-assay variability did not differ by more than 5%, and variability among mean values from separate dishes never exceeded 5%. Protein was determined with bovine serum albumin (fraction V; Sigma) as a standard (Lowry *et al.*, 1951).

Incorporation of [¹⁴C]acetate into lipids and determination of triacylglycerol content

[¹⁴C]Acetate (1 µCi; 56 Ci/mol) was added to the culture medium (2 ml of medium per dish); 2 h later, the culture medium was removed and the cells were rinsed twice at 37°C with phosphate-buffered saline, pH 7.4. Cells were solubilized by the addition of 0.8 ml of 0.1 M-NaOH. A sample (0.1 ml) was used for protein determination. The remaining solution was adjusted to acidic pH with HCl and the lipids were extracted as described by Bligh & Dyer (1959). Half of the chloroform phase was used for counting the radioactivity in the presence of 10 ml of Biofluor

(New England Nuclear Corp.). Variability between separate dishes never exceeded 6%. Triacylglycerol content was determined as described by Chernick (1969).

Immunotitrations

Anti-(fatty acid synthetase) serum was obtained through the courtesy of Dr. A. W. Alberts (Merck, Sharp and Dohme Research Laboratories, Rahway, NJ, U.S.A.). It had been obtained by immunizing a goat with homogeneous rat liver fatty acid synthetase. Partially purified anti-(fatty acid synthetase) γ-globulins were obtained as follows: solid (NH₄)₂SO₄ (250 g/l) was added to the immune serum. After 20 h at room temperature, the pellet was recovered by centrifugation (30 min at 4000 g) and rinsed twice with 1.75 M-(NH₄)₂SO₄. The pellet was finally dissolved (half of the initial volume) in 20 mM-potassium phosphate, pH 7.0, containing 150 mM-NaCl and 10 mM-mercaptoethanol. The γ-globulin fraction was extensively dialysed against the same buffer and used directly for immunotitration experiments. A non-immune γ-globulin fraction was obtained similarly. For immunotitration experiments, cells were rinsed twice at 37°C with phosphate-buffered saline, pH 7.4, and then scraped, with a rubber 'policeman', with a solution of 300 mM-potassium phosphate, pH 7.0, containing 5 mM-EDTA (0.5–1.5 ml/dish). After homogenization in a Potter–Elvehjem homogenizer (15 strokes), the homogenate, containing 0.25–0.5 mg of protein/ml, was centrifuged as described above. The immune complex was formed by treating 0.1 ml of the collected supernatant fraction with increasing concentrations of the γ-globulin immune fraction (1–100 µg of protein) and with a constant amount (150 µg) of the non-immune γ-globulin fraction, in buffer containing 300 mM-potassium phosphate, pH 7.0, 35 mM-NaCl and 0.8 mM-KCl. The final volume was 0.2 ml. After 1 h at 37°C, required for the formation of the immune complex in which fatty acid synthetase was inactive, the residual (unbound) activity was determined as described above.

Hormones and chemicals

[2-¹⁴C]Malonyl-CoA and [1-¹⁴C]acetate were products of Amersham International and Commissariat à l'Energie Atomique respectively. Culture media and foetal-calf serum were from Seromed (Munich, Germany). NADH, CoA and ATP were obtained from Boehringer Mannheim. Pig insulin (monocomponent) and pig proinsulin were products from Novo. Turkey insulin was kindly given by Professor T. L. Blundell and Dr. J. E. Pitts (Birkbeck College, London W.C.1, U.K.). Other chemicals were purchased from Sigma.

Results

Effects of insulin removal and insulin addition on marker enzymes of differentiated ob17 adipose-like cells

As shown in Fig. 1, insulin deprivation of 15 day post-confluent differentiated ob17 cells resulted quite rapidly in a decrease in the rate of [14 C]acetate incorporation into lipids (50% within 8 h after insulin

Fig. 1. *Modulation of the activities of marker enzymes of adipose conversion after insulin removal*

Confluent ob17 cells were maintained for 15 days in standard medium supplemented with 17 nM-bovine insulin and 1.5 nM-tri-iodothyronine. Culture medium was removed by aspiration and the cells were treated to eliminate insulin as described in the Experimental section. Cells were then exposed (zero time) to standard medium supplemented with 1.5 nM-tri-iodothyronine. Enzyme activities, triacylglycerol content and [14 C]acetate incorporation into lipids were determined as a function of exposure time. The 100% initial activities correspond to 960, 8.2, 12 and 2800 munits/mg of protein for glycerol 3-phosphate dehydrogenase (■), fatty acid synthetase (●), acid:CoA ligase (△) and lactate dehydrogenase (□) respectively. The triacylglycerol content (▽) was initially 0.62 μ mol/ 10^6 cells, and the incorporation of [14 C]acetate (▲) into lipids at zero time was 27500 d.p.m./2h per mg of protein. Additional experiments of [14 C]acetate incorporation into lipids were performed with cells maintained in (○) standard medium in the absence of tri-iodothyronine and insulin, (▼) standard medium in the absence of tri-iodothyronine but in the presence of insulin, or (■) standard medium in the presence of tri-iodothyronine and never exposed to insulin (17 days post-confluent cells). Although the initial specific activities may vary by as much as 50%, the pattern presented is typical of six independent experiments. From one experiment to another, each point representing the percentage of the remaining activity did not differ at any time by more than 11%.

removal). This decrease was quite parallel to those of fatty acid synthetase and glycerol 3-phosphate dehydrogenase activities, whereas that of acid:CoA ligase activity was slower but remained significant. It is noteworthy that lactate dehydrogenase, not directly related to lipogenesis, was unaffected by insulin removal. The results also showed clearly that no mobilization of intracellular triacylglycerol occurred under these conditions. At 24–48 h after insulin removal the steady-state activity of glycerol 3-phosphate dehydrogenase of cells chronically exposed for 15 days to 17 nM-insulin was almost identical with that determined on 17-day post-confluent ob17 cells never exposed to insulin (Fig. 1). This result was reproducibly observed for 9-, 13- and 17-day post-confluent cells which had been first chronically exposed to 1.7 nM- or 17 nM-insulin. In each case the activity of glycerol 3-phosphate dehydrogenase decreased after 24 h of insulin deprivation to values identical with those determined on cells that had never been exposed to insulin for corresponding periods of time (results not shown). In contrast with insulin removal, the removal of tri-iodothyronine caused no effect on the rate of [14 C]acetate incorporation into lipids (Fig. 1). This result indicated that, whereas the presence of tri-iodothyronine was essential for the expression of key enzyme markers of adipose conversion in differentiating ob17 cells (Gharbi-Chihi *et al.*, 1981; Grimaldi *et al.*, 1982), this hormone was ineffective on the expression of the same markers once the cells are differentiated.

The ability of insulin to modulate reversible effects is illustrated in Fig. 2. After addition of insulin to insulin-deprived cells, the rises in both the activity of glycerol 3-phosphate dehydrogenase and the rate of [14 C]acetate incorporation into lipids were parallel. Within 24 h both values were restored to those before insulin deprivation. The restoration of the initial acid:CoA ligase activity was also complete, but occurred more slowly. However, no significant increase in both parameters was observed within the first 4 h after addition of insulin. This initial lag period seemed to exclude short-term effects of insulin and was in favour of long-term effects of the hormone. Thus differentiated ob17 cells were able to respond to insulin removal or to insulin addition, and to adjust reversibly the activities of some key enzymes of triacylglycerol synthesis.

Dose-response curves of bovine insulin and analogues on the activity of glycerol 3-phosphate dehydrogenase and on the rate of [14 C]acetate incorporation into lipids

Confluent ob17 cells possess insulin receptors that could be down-regulated by chronic exposure to high-concentration of insulin (Grimaldi *et al.*, 1979). However, after chronic exposure to 17 nM-insulin,

Fig. 2. Recovery of the activities of enzyme markers after insulin removal followed by insulin addition

Confluent ob17 cells (a series different from that used in Fig. 1) were maintained for 14 days in standard medium supplemented with 17 nM-bovine insulin and 1.5 nM-tri-iodothyronine. Cells were then treated to remove insulin and exposed to standard medium supplemented with 1.5 nM-tri-iodothyronine. After 48 h the medium was removed and replaced (zero time) by the standard medium supplemented with 1.5 nM-tri-iodothyronine and 17 nM-insulin. Enzyme activities were determined as a function of time after insulin re-addition. The specific activities at day 14 before insulin removal were 1020 and 11.3 munits/mg of protein for glycerol 3-phosphate dehydrogenase (■) and acid:CoA ligase (△) respectively; the corresponding value for [¹⁴C]acetate incorporation into lipids (▲) determined in parallel was 35 000 d.p.m./2 h per mg of protein. Although the initial specific activities may vary by as much as 55%, the pattern presented is typical of seven independent experiments. From one experiment to another, each point representing the percentage of activity (relative to day 14) did not differ at any time by more than 15%.

significant amounts of 'high-affinity' receptors ($K_d \sim 1$ nM) remained, and therefore should allow the cells to retain their responsiveness to the hormone (results not shown).

When differentiated ob17 cells were first chronically exposed to 17 nM-insulin and then immediately exposed for 24 h to various concentrations of this hormone (Fig. 3a), the activity of glycerol 3-phosphate dehydrogenase was maximal at 17 nM-insulin and the half-maximally effective concentration was close to 1 nM. An identical curve was found for the changes in the rate of [¹⁴C]acetate incorporation into lipids. If we assume that this parameter is a good reflection of the fatty acid synthetase activity (see Fig. 1), this experiment would indicate that differentiated ob17 cells can adjust quite rapidly

the activities of two key enzymes involved in triacylglycerol synthesis.

The experiment described in Fig. 3(b) shows that ob17 cells were able, after insulin deprivation for 24 h, to respond within the next 24 h to subsequent insulin addition. The activities that were restored as a function of insulin concentration were quite similar to those observed in the experiment of Fig. 3(a). Once again, parallel changes took place for the glycerol 3-phosphate dehydrogenase activity and for the rate of [¹⁴C]acetate incorporation into lipids. The concentrations of insulin that were half-maximally and maximally effective were again found to be 1 nM and 17 nM respectively. The half-maximally effective concentrations determined in experiments described in Figs. 3(a) and 3(b) were in agreement with the K_d values for the 'high-affinity' component of insulin-receptor binding, strongly suggesting that insulin-responsiveness of ob17 cells occurred through hormone binding to insulin receptors. The experiments described in Fig. 3(c) were in agreement with this hypothesis. A rightward shift in the dose-response curves of turkey insulin, pig insulin and pig proinsulin was observed, with half-maximally effective concentrations of 0.1, 0.5 and 10 nM respectively. These values were also in agreement with the respective potency of pig insulin and pig proinsulin in displacing ¹²⁵I-labelled insulin from its binding sites (Grimaldi *et al.*, 1983).

Changes in fatty acid synthetase content after insulin removal and insulin re-addition

As mentioned above, previous studies have shown that in liver and adipose tissue the content of fatty acid synthetase, as well as of other lipogenic enzymes, undergoes changes when the rate of fatty acid biosynthesis fluctuates in response to metabolic conditions. These conditions include developmental, dietary and hormonal changes (Volpe & Vagelos, 1976). Therefore investigations were performed to test whether the rapid decrease in the specific activity of fatty acid synthetase after insulin removal was due to a decrease in the cellular content of this enzyme. The quantity of fatty acid synthetase was estimated by immunotitration. Increasing amounts of anti-(fatty acid synthetase) γ -globulins were added to a constant amount of fatty acid synthetase activity. The amount of antibody required to inactivate fatty acid synthetase completely was determined by extrapolating the linear portions of titration curves to zero fatty acid synthetase activity. As shown in Fig. 4(a), 44 μ g of the anti-(fatty acid synthetase) γ -globulin was needed to inactivate 0.078 munit of enzyme activity in insulin-exposed cells, whereas 12 μ g of the same fraction was required to inactivate 0.021 munit of enzyme activity in insulin-deprived cells. Therefore nearly equivalent amounts of antibody were needed

Fig. 3. Dose-response curves of insulin and analogues on marker enzymes

Experiments shown in (a), (b) and (c) were performed on different series of cells. (a) Confluent ob17 cells were maintained for 15 days in standard medium supplemented with 17 nM-bovine insulin and 1.5 nM-tri-iodothyronine. Cells were then treated to remove insulin (see the Experimental section) and immediately exposed to standard medium supplemented with 1.5 nM-tri-iodothyronine and increasing concentrations of bovine insulin as indicated. After 24 h, the activity of glycerol 3-phosphate dehydrogenase (■) and the incorporation of [¹⁴C]acetate into lipids (▲) were determined; the 100% activity was obtained at 17 nM-insulin and corresponds to 930 munits/mg of protein for glycerol 3-phosphate dehydrogenase and to 32 500 d.p.m. of [¹⁴C]acetate incorporated into lipids/2 h per mg of protein. (b) Confluent ob17 cells were maintained for 13 days in standard medium supplemented with 17 nM-insulin and 1.5 nM-tri-iodothyronine. Cells were then treated to remove insulin and maintained in standard medium supplemented with 1.5 nM-tri-iodothyronine. Then 24 h later, bovine insulin was added at increasing concentrations, as indicated. The activities were determined 24 h after insulin addition. The 100% activity corresponds to 960 munits/mg of protein for glycerol 3-phosphate dehydrogenase (■) and to 26 500 d.p.m./2 h per mg of protein for [¹⁴C]acetate incorporation into lipids (▲). (c) Confluent ob17 cells were maintained for 15 days in standard medium supplemented with 17 nM-bovine insulin and 1.5 nM-tri-iodothyronine. After treatment to remove insulin, cells were maintained for 24 h in standard medium supplemented with 1.5 nM-tri-iodothyronine. Subsequently, turkey insulin (▽), pig insulin (▲) or pig proinsulin (△) was added at increasing concentrations, as indicated. [¹⁴C]Acetate incorporation into lipids was determined 24 h after peptide addition. The 100% values are 46 370, 42 390 and 43 160 d.p.m./2 h per mg of protein for turkey insulin-, pig insulin- and pig proinsulin-treated cells respectively. Although the maximal specific activities may vary by as much as 47%, the patterns presented are typical of six (a), four (b) and two (c) independent experiments. From one experiment to another, each point representing the percentage of the maximal activity did not differ at any concentration by more than 10%.

to neutralize a given amount of enzyme activity (1 munit of enzyme activity neutralized by 0.568 mg of antibody). It is clear that insulin removal resulted in a decrease in the cellular content of fatty acid synthetase rather than in a de-activation of pre-existing active enzyme molecules. As illustrated by the curves in Fig. 4(b), insulin re-addition to cells previously deprived of insulin for 48 h led within 48 h to an almost complete restoration of the initial enzyme content. Similarly to the experiment described in Fig. 4(a), nearly equivalent amounts of antibody were required to neutralize a given amount of fatty acid synthetase activity (1 munit of enzyme activity neutralized by 0.493 mg of antibody).

These results strongly suggest that the decrease (after insulin removal) or the increase (after insulin

re-addition) in the specific activity of fatty acid synthetase were respectively due to the decrease and to the increase in the cellular content of the enzyme.

Discussion

Differentiated ob17 cells, which possess both the morphological and the biochemical properties of mature adipocytes (Ailhaud *et al.*, 1983), are able to respond within hours to insulin (Figs. 1 and 2). The rapid decrease observed in the fatty acid synthetase and glycerol 3-phosphate dehydrogenase activities after insulin removal is striking and can be postulated to occur *in vivo*, i.e. during starvation. This phenomenon is directly due to insulin, and it is fully reversible after hormone addition. The continuous

Fig. 4. Immunotitration of fatty acid synthetase in insulin-deprived and insulin-exposed ob17 differentiated cells (a) Insulin removal: confluent cells were maintained for 10 days in standard medium supplemented with 17 nM-insulin, and 1.5 nM-tri-iodothyronine. Then cells were treated to remove insulin as described in the Experimental section and maintained for 48 h either in standard medium supplemented with 1.5 nM-tri-iodothyronine (insulin-deprived cells) or in standard medium supplemented with 1.5 nM-tri-iodothyronine and 17 nM-insulin (control cells). In both cases, a supernatant fraction was used as described in the Experimental section for the immunotitration of fatty acid synthetase. Points on the curves represent means of duplicate determinations performed on a supernatant fraction from two pooled dishes. Variations between duplicate determinations did not exceed 5%. The 100% residual activity corresponds to 0.078 and 0.021 munit of fatty acid synthetase for control (●) and insulin-deprived cells (○) respectively. The specific-activity values of fatty acid synthetase were 4.4 and 1.9 munits/mg of protein for control and insulin-deprived cells respectively. The curves are representative of two independent experiments. (b) Insulin removal followed by insulin addition: confluent cells from a series different from that used in (a) were maintained for 10 days in standard medium supplemented with 17 nM-insulin and 1.5 nM-tri-iodothyronine. They were first treated to remove insulin as described in the Experimental section, then maintained for 48 h in insulin-deprived medium (1.5 nM-tri-iodothyronine alone) and finally shifted for 48 h to insulin-containing medium (1.5 nM-tri-iodothyronine and 17 nM-insulin). Control cells were maintained for 14 days after confluence in standard medium supplemented with 17 nM-insulin and 1.5 nM-tri-iodothyronine. Points on the curves represent means of duplicate determinations performed on a supernatant fraction from three pooled dishes from a single experiment. Variations between duplicate determinations did not exceed 5%. The 100% residual activity corresponds to 0.096 and 0.07 munit of fatty acid synthetase for control (●) and experimental cells (○) respectively. The specific-activity values were 4.3 and 3.98 munits/mg of protein for control and experimental cells respectively.

presence of insulin is necessary, and ob17 adipose-like cells adjust their steady-state activities of triacylglycerol-pathway enzymes as a function of insulin concentrations. It must be stressed that these responses occur within physiological concentrations of insulin (Fig. 3).

The direct modulation of fatty acid synthetase by insulin is in agreement with previous results obtained *in vivo* and *in vitro*. On one hand, studies *in vivo* on rat adipose tissue had shown that fatty acid synthetase was dependent on insulin for modulation of its activity (Volpe & Vagelos, 1974) and that there was a correlation between plasma insulin concentration and fatty acid synthetase activity (Bruckdorfer *et al.*, 1972). On the other hand, studies *in vitro* on cultured Chang liver cells had shown that the continuous presence of insulin was required for maintenance of elevated fatty acid synthetase activity, and the half-maximally effective concentration of insulin was found to be 2 nM (Alberts *et al.*, 1974).

The long-term modulation of fatty acid synthetase activity by insulin in ob17 adipose-like cells is correlated with parallel modulation of the rate of [^{14}C]acetate incorporation into lipids, suggesting that, under these conditions, changes in the enzyme activity might be rate-limiting in fatty acid biosynthesis.

Immunotitration experiments demonstrate that changes in fatty acid synthetase activity reflect parallel changes in the enzyme content (Fig. 4). The immunotitration procedure used in this study provides relative, but not absolute, measurement of mass. However, since the specific-activity values of the cytosolic fraction are quite similar to those found in differentiated 3T3-L1 and 3T3-F442A cells, in which fatty acid synthetase content accounts for 2.5–5% of the cytosolic proteins (Mackall *et al.*, 1976; Ahmad *et al.*, 1979; Student *et al.*, 1980; Weiss *et al.*, 1980; Spiegelman & Green, 1980, 1981), it is likely that similar amounts are present in differentiated ob17 cells.

So far, the direct and long-term modulation of the glycerol 3-phosphate dehydrogenase activity by insulin has not been reported in adipose cells. In 3T3-F442A cells the activity of this enzyme is not affected by insulin (Spiegelman & Green, 1980), in contrast with ob17 cells, in which it is under all conditions co-ordinated with the rate of fatty acid synthesis. The co-ordinate nature of the responses suggests that the effects of insulin occur through a common intermediary step after insulin binding. As shown in the present paper and as previously proposed (Grimaldi *et al.*, 1983), long-term insulin effects occur most probably after binding to insulin receptors. In conclusion, differentiated ob17 adipose-like cells represent a cellular model exquisitely sensitive to metabolic modulation of lipogenesis and triacylglycerol synthesis by insulin.

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