Hormonal Regulation of Stearoyl Coenzyme A Desaturase Activity and Lipogenesis during Adipose Conversion of 3T3-L1 Cells*

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During differentiation of 3T3-L1 cells to adipocytes the desaturation of exogenous [14C]palmitate and of de novo synthesized fatty acids from exogenous [14C]acetate to monounsaturated fatty acids increased by 5.9- and 255-fold, respectively. Under similar conditions, fully differentiated 3T3-L1 adipocytes showed a maximal induction of 100-, 63-, and 50-fold, respectively, in the activities of stearoyl-CoA desaturase, fatty acid synthetase, and malic enzyme. The extent of differentiation into adipocytes and the increase in enzymatic activities occurred more slowly when the differentiation was induced by insulin alone than when differentiation was induced by dexamethasone, 1-methyl-3-isobutylxanthine, and insulin. In one experiment a 30-fold induction of overall stearoyl-CoA desaturation was compared with a 92-fold increase in the activity of terminal component of the desaturase system on differentiation of 3T3-L1 cells. The activities of NADH-ferricyanide reductase, NADH-cytochrome c reductase, and the content of cytochrome b5 increased by 3.4-, 2.3-, and 6.6-fold, respectively, on differentiation of 3T3-L1 cells. However, reconstitution of the stearoyl-CoA desaturation in various preparations of microsomes with excess NADH-cytochrome b5 reductase and cytochrome b5 indicated that the changes in the reductase activity and cytochrome b5 content are not influencing the expression of overall stearoyl-CoA desaturation in microsomes. The induction of stearoyl-CoA desaturation during adipose conversion of 3T3-L1 cells is primarily due to an increase in the activity of Δ9 terminal desaturase. Biotin deficiency in 3T3-L1 adipocytes inhibited de novo fatty acid synthesis from [14C]acetate but did not inhibit phenotypic expression of lipid accumulation as well as activities of stearoyl-CoA desaturase and fatty acid synthetase, suggesting that continued synthesis of saturated fatty acid substrate is not required for the induction of stearoyl-CoA desaturase activity. The induction of stearoyl-CoA desaturase and fatty acid synthetase activities as well as enhanced synthesis of fatty acids and sterols during 3T3-L1 cell differentiation is inhibited by anti-insulin serum, and this inhibition is relieved by the addition of exogenous insulin. These results indicate that insulin supports phenotypic expression as well as the induction of lipogenesis and the activities of stearoyl-CoA desaturase and fatty acid synthetase during adipose conversion of 3T3-L1 cells.

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The murine 3T3-L1 cell line developed by Green and coworkers (1-4) has provided an excellent model system for studying the molecular mechanisms involved in the hormonal and metabolic regulation of major lipogenic enzymes. Exponentially growing and preconfluent cells exhibit biochemical and morphological properties of fibroblasts (1, 5), but confluent cells differentiate into adipocytes, either spontaneously (2) or after treatment with a combination of hormones, drugs, and nutrients (3, 5-8). Rapid and uniform differentiation of 3T3-L1 cells is achieved by priming the cells with 0.5 mM 1-methyl-3-isobutylxanthine and 0.25 μM dexamethasone for 24 h and then allowing a period of 5-10 days in drug-free growth medium, preferably supplemented with insulin, for phenotypic expression. During adipocyte conversion, 3T3-L1 cells exhibit a coordinate rise in almost all of the enzymes of de novo fatty acid synthesis (5, 6, 9-12) and triacylglycerol synthesis (13, 14). The differentiation-dependent increase in the activities of acetyl-CoA carboxylase and fatty acid synthetase, the two enzymes that participate in the de novo synthesis of saturated long chain fatty acids (palmitate and stearate), results from an increase in the content of the two enzymes (6, 9-12). The increase in the fatty acid synthetase content is due to a corresponding increase in its rate of synthesis (10, 11, 15, 16).

Previous studies on adipose conversion in 3T3-L1 cells have essentially emphasized overall lipogenesis and de novo synthesis of saturated fatty acids. Oleic acid constitutes about 40% of the total fatty acids in the mouse depot fat. Furthermore, palmitoleate and oleate, which constitute 92 and 36%, respectively, of the total cellular fatty acids in 3T3-L1 cells, increase 8-fold during adipose conversion, although not more than 22% of the palmitoleate and oleate in differentiated cells can be directly derived from the culture medium. This result suggests that during adipose conversion of 3T3-L1 cells, the majority of the cellular palmitoleate and oleate must be synthesized, de novo, or made by desaturation of exogenous palmitate and stearate. The present study reports on the induction of microsomal stearoyl-CoA desaturase activity which is responsible for the cellular synthesis of palmitoleate and oleate during differentiation of 3T3-L1 cells. Addition of anti-insulin antibodies to the culture medium inhibits cell differentiation, lipogenesis, and induction of stearoyl-CoA desaturase and fatty acid synthetase enzymes in 3T3-L1 cells. Excess insulin in the culture medium reverses the effects of anti-insulin serum suggesting that insulin is essential for induction of lipogenesis, stearoyl-CoA desaturase, fatty acid synthetase, and phenotypic expression.

**EXPERIMENTAL PROCEDURES**

_Culture Conditions—_Culture dishes (60 mm) were inoculated with 1-2 × 10⁶ 3T3-L1 cells and grown to confluence in 3 ml of DME medium containing 10% fetal calf serum, 50 μg of streptomycin/ml, 100 units of penicillin/ml, 1 μg of insulin/ml, 10⁻⁷ M dexamethasone, and 10⁻⁵ M 1-methylisobutylxanthine, and then allowed to differentiate until confluent. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The abbreviation used is: DME medium, Dulbecco's modified Eagle's medium.
and 50 micromg of penicillin/ml in a humified atmosphere of 5% CO\textsubscript{2} and 85% air at 37 °C. Cultures were fed every 2 days with fresh medium during growth and differentiation unless otherwise indicated. A day after confluence, the cells were fed with medium supplemented with 0.5 mM 1-methyl-3-isobutylxantine and 25 μM dexamethasone to induce differentiation (6, 10). The culture medium was withdrawn 72 h after feeding in order to synchronize the proliferative potential of the nonpassaged culture medium supplemented with 1.6 μM insulin. By 12–15 days after confluence, 90% of the cells in monolayer expressed the adipocyte phenotype. In some experiments differentiation was also induced by treating cells with culture medium supplemented with 1.6 μM insulin alone. Undifferentiated 3T3-L1 cells were maintained in an identical manner but in the absence of drugs and insulin. Since the frequency of adipose conversion decreased after about 10–15 passages, stock cultures for inoculation were derived before 5 passages.

In some experiments biotin-deficient medium was used to produce biotin deficiency in cultures. For this purpose, DME medium without biotin but containing 10% fetal calf serum was incubated with 0.1 μM avidin for 30 min at 37 °C to facilitate binding of biotin that may be present in the serum (13). 3T3-L1 cells were inoculated in normal culture medium and allowed to attach to the substratum for 24 h. At this point the culture medium was replaced with biotin-deficient medium to induce and sustain differentiation.

Insulin-deficient culture medium was prepared by binding serum insulin to anti-insulin guinea pig serum (Cappel Laboratories, PA). Different amounts of anti-insulin serum and the required amount of fetal calf serum to make up to 10% serum were added to DME medium. 3 T3-L1 cells were incubated for 30 min at 37 °C to facilitate binding of insulin and insulin-like cross-reacting material in the culture medium. The insulin-deficient culture medium was then used for feeding the confluent 3T3-L1 cells.

In order to measure the extent of differentiation, cell monolayer was fixed in phosphate-buffered formalin (10%) for 24 h, rinsed with water followed by 70% ethanol, and stained with oil Red O solution (6 parts of saturated oil red O dye in isopropanol + 4 parts of water) for 15 min. Excess stain was removed by washing with 70% ethanol until no more dye diffused out. The stained cells were washed finally with phosphate-buffered saline. The cell monolayer was then incubated for 5 min with 1.5 ml of 4% Nonidet P-40 in isopropanol which dissolved stained oil droplets. The absorbance of the dye-triglyceride complex was measured at 520 nm after suitable dilution. Typically the absorbance in extracts of fully differentiated cells is 40 times higher than that of control cells.

Preparation of Cell Extract—Cells in monolayer were washed twice with phosphate-buffered saline, pH 7.4, and once with 10 mM Tris-HCl, pH 7.4, containing 1 mM dithiothreitol and 0.25 mM sucrose. Cells were removed by scraping with a rubber policeman and suspended in ice-cold buffer containing 10 mM Tris-HCl, pH 7.4, 1 mM dithiothreitol and 0.25 mM sucrose. The cell monolayer was then incubated for 5 min with 1.5 ml of 4% Nonidet P-40 in isopropanol which dissolved stained oil droplets. The absorbance of the dye-triglyceride complex was measured at 520 nm after suitable dilution. Typically the absorbance in extracts of fully differentiated cells is 40 times higher than that of control cells.

Enzyme Assays—Fatty acid synthase activity was assayed in the soluble cytosolic fraction by measuring the NADPH-dependent incorporation of [1-14C]palmitoyl-CoA (2 μCi/μmol) into fatty acids at 37 °C as described previously (19). The enzyme activity is expressed as nanomoles of malonyl-CoA incorporated into fatty acids per min.

Malate dehydrogenase activity was measured in the soluble fraction according to the method of Wise and Ball (20). The enzyme activity was assayed at 37 °C by measuring the rate of malate-dependent reduction of NADPH at 340 nm in a Gilford spectrophotometer. The reaction mixture contained 45 mM Tris-HCl, pH 7.4, 0.56 mM sodium malate, 4.5 mM MgCl\textsubscript{2}, 10 mM NADPH, and 75–100 μg of protein in a final volume of 0.5 ml.

Microsomal stearoyl-CoA desaturase activity was assayed by the method of Jones et al. (21). Microsomes (0.1–0.5 mg of protein) were incubated in a final volume of 0.5 ml with 10 nmol of [1-14C]stearoyl-CoA (5 μCi/μmol) and 100 nmol of NADH in 60 mM potassium phosphate buffer, pH 7.2, for 20 min at 37 °C. The methyl esters of [1-14C]stearate and [1-14C]palmitate were separated by thin layer chromatography (22). The radioactivity was quantitated using a liquid scintillation counter. Microsomal NADH-ferricyanide reductase and NADH-cytochrome c reductase activities were determined as described by Wilson et al. (22). Cytochrome b content of microsomes was determined from the NADH-reduced minus oxidized spectra using 340 nm of 185 m\textsuperscript{-1} cm\textsuperscript{-1} between 424 and 409 in an Aminco DW-2 spectrophotometer (22, 23).

The Δ terminal desaturase activity in microsomes was assayed by measuring the rate of stearoyl-CoA-stimulated oxidation of reduced cytochrome c. The reaction mixture contained 50 mM Tris-HCl, pH 7.2, 200 μM freshly prepared Na\textsubscript{2}S and 1 mg of microsomal protein in a final volume of 1 ml. Microsomal cytochrome b\textsubscript{5} was reduced by 0.5 nmol of NADH, and the reoxidation was recorded by following the absorbance difference between 424 and 409 nm in an Aminco DW-2 spectrophotometer in the dual wavelength mode. When the reoxidation of cytochrome b\textsubscript{5} was complete, 10 nmol of stearoyl-CoA followed by 0.5 nmol of NADH were added, and the reoxidation was recorded again. The rate of stearoyl-CoA-stimulated oxidation of reduced cytochrome b\textsubscript{5} was calculated as described by Stritmatter et al. (24).

All enzyme assays were done in duplicate on cell extracts isolated from four pooled culture dishes. The enzyme activities of duplicate samples did not differ by more than 5%. Enzyme activities are expressed as units per culture dish, or when appropriate, as units/mg of microsomal or cytosolic protein.

Reconstitution of Stearyl-CoA Desaturase Activity of 3T3-L1 Cell Microsomes—Since the stearyl-CoA desaturase is a multicomponent system, stearyl-CoA desaturation in 3T3-L1 cell microsomes was reconstituted in detergent extracts of 3T3-L1 cell microsomes supplemented with chick embryo liver microsomes, which lack stearyl-CoA desaturase activity but provide full complement of NADH-cytochrome c reductase activity and cytochrome b\textsubscript{5} (22). The enzyme activity was reconstituted according to the method of Joshi et al. (25). Briefly, varying amounts of 3T3-L1 cell microsomes (40 to 150 μg of protein) were incubated for 5 min at 4 °C with 400 μg of hepatic microsomes from 20-day-old chick embryos in a final volume of 50 μl containing 1% Triton X-100. The stearyl-CoA desaturase activity in this reconstituted extract is then assayed at 37 °C in a final volume of 0.5 ml by measuring the NADH-dependent desaturation of [1-14C]stearoyl-CoA as described above.

Incorporation of [1-14C]Acetate and [1-14C]Palmitate into Fatty Acids in 3T3-L1 Cells—Cells were incubated with 10 μCi of [1-14C]acetate (57.7 mCi/mmol) or 2 μCi of [1-14C]palmitate (57.2 mCi/mmol) added to 3 ml of culture medium for 1 h at 37 °C. The medium was then removed and the cultures were washed 3 times with phosphate-buffered saline. The cells were suspended in 1 ml of phosphate-buffered saline and saponified for 30 min in a boiling water bath after adding 1 ml of 20% KOH and 1 ml of ethanol. Sterols were extracted three times with 3 ml of pentane. Fatty acids were then extracted with 3 × 3 ml of pentane after acidification of the alkaline extract with 4 M H\textsubscript{2}SO\textsubscript{4}. Stearic acid (150 μg) and oleic acid (150 μg) were added as carrier, and the fatty acids were methylated with diazomethane. The methyl esters of saturated and monounsaturated fatty acids were separated on AgNO\textsubscript{3}-impregnated Silica Gel H thin layer plates (22). The spots corresponding to saturated and monounsaturated fatty acid esters were scraped from the plates into counting vials containing toluene-based scintillation fluid, and radioactivity was measured.

Total lipids were extracted with chloroform:methanol (2:1) according to Radin (26). In some experiments total lipids were saponified with KOH, and the sterols and fatty acids were separately extracted as described above.

RESULTS

The desaturation capacity of undifferentiated and differentiated 3T3-L1 cells was calculated by measuring the conversion of exogenous [1-14C]palmitate to monounsaturated fatty acids and by de novo synthesis of monounsaturated fatty acids from [1-14C]acetate. As shown in Table I, desaturation of exogenous palmitoyl-CoA to monoene fatty acids is 5.9-fold higher than that in undifferentiated cells. However, the desaturation of de novo synthesized fatty acids from [1-14C]acetate in differentiated cells was 255-fold greater than that in undifferentiated cells. In undifferentiated 3T3-L1 cells only 11% of de novo synthesized fatty acids are monounsaturated whereas 43% of the synthesized fatty acids are monounsaturated in differentiated cells. In comparison to the desaturation of exogenous fatty acids, a much higher proportion of the de novo synthesized fatty acids is desaturated in 3T3-L1 cells. This
Insulin Regulation of Stearyl-CoA Desaturase in 3T3-L1 Cells

The observation suggests that the de novo synthesized fatty acids are perhaps handled differently than exogenous fatty acids due to metabolic compartmentation. A preferential association of an active form of acetyl-CoA carboxylase with microsomes (27) suggests that palmitate and stearate synthesized, de novo, may indeed be more accessible for desaturation by the microsomal Δ⁶ desaturase. Nonetheless, the results with [¹⁴C]palmitate and [¹⁴C]acetate indicate that the desaturation capacity is markedly elevated upon adipose conversion in 3T3-L1 cells.

An increase in the formation of monounsaturated fatty acids was corroborated by an increase in the stearyl-CoA desaturase activity during adipose conversion of 3T3-L1 cells (Table II). This induction of enzyme activity during cell differentiation was also shared by two other lipogenic enzymes, fatty acid synthetase and malic enzyme. The extent of differentiation into adipose cells and the increase in enzyme activities occurred more slowly when the differentiation was induced by insulin alone than when differentiation was induced by dexamethasone, methylisobutylxanthine, and insulin. In control cells which are not exposed to exogenous insulin, the enzyme activities varied with different batches of serum. The values reported in this paper were obtained with different batches of serum. The values in parentheses represent specific activities expressed as pmol/min/mg of microsomal protein. The extent of phenotypic differentiation as well as induction of enzyme activities in confluent cells increased with the time of incubation with insulin or dexamethasone, methylisobutylxanthine, and insulin. Adipose conversion by treatment with dexamethasone, methylisobutylxanthine, and insulin resulted in an increase of cell protein by 2- to 3-fold, although the number of cells remained constant. At the end of 15 days of confluence the specific activities (units/mg of microsomal or cytosolic protein) of stearyl-CoA desaturase, fatty acid synthetase, and malic enzyme increased by 12.7-, 16.4-, and 12.6-fold, respectively, in dexamethasone, methylisobutylxanthine-, and insulin-treated cells (Table II). The increase in the specific activities of malic enzyme and fatty acid synthetase is comparable to that reported by other investigators (11, 32). A noteworthy point is that the induction of malic enzyme activity by insulin alone is much smaller than the corresponding insulin induction of fatty acid synthetase and stearyl-CoA desaturase activities.

Since the overall stearyl-CoA desaturation involves the participation of NADH-cytochrome b₅ reductase, cytochrome b₅, and Δ⁶ terminal desaturase protein (23, 24), the component activities of the microsomal desaturase system were measured to determine whether there are corresponding changes in these activities during 3T3-L1 cell differentiation. The microsomal activities of NADH-ferricyanide reductase (a measure of the flavoprotein), NADH-cytochrome c reductase (a measure of the flavoprotein and cytochrome b₅), and the content of cytochrome b₅ increased in differentiated cells by 3.4-, 2.3-, and 6.6-fold, respectively, whereas the stearyl-CoA desaturase activity increased by 30-fold (Table III). The increases in the total activities of the two reductases as well as in the content of cytochrome b₅ resulted primarily from an increase in the content of endoplasmic reticulum as reflected by a 4.5-fold increase in microsomal protein in differentiated cells. Thus, in differentiated cells the specific activities (units/mg of microsomal protein) of the two reductases decreased by 21 and 46%, and the specific content (pmol/mg of microsomal protein) of cytochrome b₅ decreased by 42% as compared to those in microsomes from undifferentiated cells. A 30-fold increase in the stearyl-CoA desaturase per dish during adipose conversion correlated reasonably with a 32-fold increase in the Δ⁶ terminal desaturase activity as measured by the stearyl-CoA-dependent rate of reoxidation of reduced cytochrome b₅. These results lead us to conclude that the increase in stearyl-CoA desaturase activity during 3T3-L1 cell differentiation is due to an increase in the activity of the terminal desaturase component.

The specific activities of NADH-ferricyanide reductase and

Table I

Incorporation of [¹⁴C]acetate and [¹⁴C]palmitate into 3T3-L1 cells during adipose differentiation

After confluence, the cells were either allowed to differentiate or remain undifferentiated for 15 days as described under "Experimental Procedures." Cells were incubated for 1 h with either 2 μCi of [¹⁴C]palmitate (57.9 mCi/mmol) or 10 μCi of [¹⁴C]acetate (57.7 mCi/mmol).

<table>
<thead>
<tr>
<th>Treatment after confluence</th>
<th>Stearyl-CoA desaturase</th>
<th>Fatty acid synthetase</th>
<th>Malic enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Monoenic acid</td>
<td>Total fatty</td>
</tr>
<tr>
<td></td>
<td>acids</td>
<td>acids</td>
<td>acids</td>
</tr>
<tr>
<td>Undifferentiated cells</td>
<td>955</td>
<td>39</td>
<td>23</td>
</tr>
<tr>
<td>Differentiated cells</td>
<td>1738</td>
<td>231</td>
<td>1527</td>
</tr>
</tbody>
</table>

Table II

Changes in activities of stearyl-CoA desaturase, fatty acid synthetase, and malic enzyme during adipocyte differentiation

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Stearyl-CoA desaturase</th>
<th>Fatty acid synthetase</th>
<th>Malic enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment after confluence</td>
<td>7°</td>
<td>10°</td>
<td>15°</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Insulin</td>
<td>10</td>
<td>50</td>
<td>90</td>
</tr>
<tr>
<td>DEX + MIX*</td>
<td>(32)</td>
<td>(162)</td>
<td>(489)</td>
</tr>
<tr>
<td>DEX + MIX + insulin</td>
<td>(94)</td>
<td>(120)</td>
<td>(406)</td>
</tr>
</tbody>
</table>

*These numbers represent days after confluence.
* The values in parentheses represent specific activities expressed as pmol/min/mg of microsomal or cytosolic protein.
* DEX, dexamethasone; MIX, methylisobutylxanthine.
NADH-cytochrome c reductase in microsomes of 3T3-L1 adipocytes are almost similar to the specific activities in rat liver microsomes, whereas the concentration of cytochrome c52b, is 5 times lower than that in rat liver microsomes (28, 29). This raised the possibility that the content of cytochrome bs may be limiting the overall desaturation in 3T3-L1 adipocytes. In order to test this possibility, the stearyl-CoA desaturase activity in 3T3-L1 cell microsomes was reconstituted by supplementation with chick embryo liver microsomes which provided additional NADH-cytochrome bs reductase and cytochrome bs but lacked any endogenous terminal desaturase (23, 25). As shown in Fig. 1, microsomal preparations from both differentiated and undifferentiated cells showed stearyl-CoA desaturase activity in the reconstituted assay. The reconstituted stearyl-CoA desaturase increased with increasing concentration of terminal desaturase from 3T3-L1 microsomes, and at saturating amounts of terminal desaturase (120-150 µg of 3T3-L1 adipocyte microsomes) in the presence of fixed amounts of other components of the desaturation system, a maximal amount of functional complex (and, therefore, a saturating stearyl-CoA desaturation) is attained. At the nonsaturating and linear portion of the curve (90 µg of microsomal protein), it is evident that the 3T3-L1 adipocyte microsomes contain 10-fold higher reconstituted stearyl-CoA desaturase activity than undifferentiated 3T3-L1 cell microsomes. This increase in the reconstituted activity is similar to the 10-fold increase in activity observed with unsupplemented microsomes from differentiated and undifferentiated 3T3-L1 cells. This result indicates that supplementation of differentiated 3T3-L1 cell microsomes with NADH-cytochrome bs reductase and cytochrome bs does not increase stearyl-CoA desaturase activity and suggests that the normal reductase activity and lower cytochrome bs concentration are not limiting the expression of overall stearyl-CoA desaturase in 3T3-L1 adipocyte microsomes.

Stearoyl-CoA desaturase has been implicated as a regulatory enzyme in lipogenesis, since stearyl-CoA desaturase is the rate-limiting step in the overall de novo synthesis of unsaturated fatty acids from acetyl-CoA in animal tissues (30, 31). It is, therefore, of considerable interest to investigate whether inhibition of de novo fatty acid synthesis affects stearyl-CoA desaturase activity in 3T3-L1 adipocytes. Biotin-deficient conditions produce rapid inhibition of de novo fatty acid synthesis, presumably through the lack of acetyl-CoA carboxylase and pyruvate carboxylase activities, without affecting phenotypic expression during 3T3-L1 cell differentiation (13, 15, 32, 33). We used avidin-supplemented biotin-free culture medium to determine the effect of biotin deficiency on differentiation, lipogenesis, and activity of stearyl-CoA desaturase and fatty acid synthetase. As shown in Table IV, biotin deficiency resulted in 77 and 73% decrease in the incorporation of [14C]acetate into fatty acids in 7- and 11-day confluent 3T3-L1 adipocytes, respectively, whereas phenotypic expression as measured by the intensity of staining with oil Red O and increase in cell protein was not affected. The absence of complete inhibition of fatty acid synthesis in avidin-treated 3T3-L1 cells may reflect conservation of intracellular biotin for metabolic processes. There is a small decrease in the proportion of monounsaturated fatty acids synthesized by cultures differentiated in biotin-deficient medium in comparison to cultures differentiated in normal medium. However, induction of both stearyl-CoA desaturase and fatty acid synthetase activities during adipogenesis is not inhibited but is rather slightly elevated by biotin deficiency (Table V). The

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**Table III**

Component activities of stearyl-CoA desaturase system in 3T3-L1 cells during differentiation

<table>
<thead>
<tr>
<th>Undifferentiated cells</th>
<th>Differentiated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stearyl-CoA desaturase, pmol/min/dish</td>
<td>10 (43)</td>
</tr>
<tr>
<td>NADH-ferricyanide reductase, nmol/min/dish</td>
<td>480 (2,077)</td>
</tr>
<tr>
<td>NADH-cytochrome c reductase, nmol/min/dish</td>
<td>150 (649)</td>
</tr>
<tr>
<td>Cytochrome bs, pmol/dish</td>
<td>8 (35)</td>
</tr>
<tr>
<td>Δ Terminal desaturase activity, pmol of cytochrome bs oxidized/s/dish</td>
<td>0.05 (0.22)</td>
</tr>
</tbody>
</table>

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**Table IV**

Effect of biotin deficiency on [14C]acetate incorporation into fatty acids during 3T3-L1 cell differentiation

<table>
<thead>
<tr>
<th>Treatment after confluence</th>
<th>7 days after confluence</th>
<th>11 days after confluence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell protein/dish</td>
<td>[14C] Acetate incorporation in total fatty acids</td>
<td>% cpm in monoenic acid</td>
</tr>
<tr>
<td>mg</td>
<td>pmol x 10^6</td>
<td>mg</td>
</tr>
<tr>
<td>None</td>
<td>0.44</td>
<td>22</td>
</tr>
<tr>
<td>DEX, MIX,* and insulin</td>
<td>0.81</td>
<td>962</td>
</tr>
<tr>
<td>DEX, MIX, avidin, and insulin in biotin-deficient medium</td>
<td>9.74</td>
<td>222</td>
</tr>
</tbody>
</table>

*DEX, dexamethasone; MIX, methylisobutylxanthine.
results suggest that the newly synthesized saturated fatty acid substrates is not an inducer of the Δ⁹ terminal desaturase activity in 3T3-L1 adipocytes.

The role of insulin in the induction of lipogenesis and stearoyl-CoA desaturase activity during 3T3-L1 cell differentiation was then examined. Initial studies were carried out to determine the dose of anti-insulin serum required in the culture medium to inhibit differentiation and lipogenesis. Since the serum in the culture medium contains some level of insulin, treatment of the confluent cells with dexamethasone and methylisobutylxanthine resulted in 6.8- and 10.9-fold stimulation of total fatty acid and monoenoic fatty acid synthesis, respectively, over control cells and 25% differentiation which is 4-fold lower than that resulting from dexamethasone, methylisobutylxanthine, and insulin treatment (Table VI). In comparison to undifferentiated control cells, dexamethasone, methylisobutylxanthine, and insulin treatment produced maximal differentiation and resulted in 40-, 82- and 8-fold stimulation of synthesis of total fatty acids, monounsaturated fatty acids, and sterol (data not shown), respectively, as measured by [14C]acetate incorporation (Table VI). In a separate experiment, the rate of incorporation of H₂O into fatty acids and sterols was enhanced by 38- and 14-fold, respectively, in fully differentiated cells. The rate of H₂O incorporation accurately reflects the rates of syntheses of fatty acids and sterols because changes in the pool size of the tracer are avoided with ¹⁸O₂. Addition of anti-insulin guinea pig serum at 0.5% concentration to dexamethasone- and methylisobutylxanthine-treated cultures resulted in 95 and 70% inhibition of differentiation and fatty acid synthesis, respectively (Table VI). Further increase in the concentration of anti-insulin serum up to 7.5% produced complete inhibition of differentiation and fatty acid synthesis. Addition of preimmunized guinea pig serum up to 5% concentration to dexamethasone- and methylisobutylxanthine-treated cultures did not affect the induction of differentiation and fatty acid synthesis in 3T3-L1 adipocytes. In comparison to control cells, a significant decrease in the cell protein content is observed on treatment with anti-insulin serum suggesting that serum insulin is required for positive nitrogen balance.

The inhibitory effect of anti-insulin serum on the synthesis of saturated and unsaturated fatty acids and on the activities of fatty acid synthetase and stearoyl-CoA desaturase can be reversed by the addition of insulin (Table VII). The increase in the rate of fatty acid synthesis and activities of stearoyl-CoA desaturase and fatty acid synthetase as well as the extent of differentiation resulting from dexamethasone, methylisobutylxanthine, and serum insulin treatment of 3T3-L1 cell cultures was inhibited by the addition of 0.5% anti-insulin serum. The inhibitory effect of anti-insulin serum on growth and differentiation was reversed by the addition of 1 µg or 10 µg of insulin/mL. The inhibitory effect of anti-insulin serum on the activities of stearoyl-CoA desaturase and fatty acid synthetase was reversed by the addition of insulin.

### Table V

**Effect of biotin deficiency on induction of stearoyl-CoA desaturase and fatty acid synthetase activities in 3T3-L1 cells**

<table>
<thead>
<tr>
<th>Treatment after confluence</th>
<th>Steroyl-CoA desaturase</th>
<th>Fatty acid synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/min/dish</td>
<td>pmol/min/dish</td>
</tr>
<tr>
<td>None</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>DEX, MIX and insulin</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DEX, MIX, avidin, and insulin in bio-tin-deficient medium</td>
<td>83</td>
<td>250</td>
</tr>
</tbody>
</table>

* Numbers represent days after confluence.
* DEX, dexamethasone; MIX, methylisobutylxanthine.

### Table VI

**Effect of anti-insulin serum on 3T3-L1 cell differentiation into adipocytes and on [¹⁴C]acetate incorporation into fatty acids**

After confluence, culture dishes were treated with dexamethasone (DEX) and methylisobutylxanthine (MIX) for 72 h. Dishes were then fed with either medium containing 10 µg of insulin/ml or medium containing different concentrations of anti-insulin serum for 10 days. Cultures were pulsed with [¹⁴C]acetate (10 µCi/dish) for 1 h.

<table>
<thead>
<tr>
<th>Treatment after confluence</th>
<th>Extent of differentiation</th>
<th>Cell protein</th>
<th>[¹⁴C]Acetate incorporation into fatty acids</th>
<th>Total fatty acids</th>
<th>Monoenoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>µg/dish</td>
<td>cpm × 10⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>2</td>
<td>451</td>
<td>21.8</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>DEX and MIX</td>
<td>25</td>
<td>437</td>
<td>150</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>DEX, MIX, and insulin</td>
<td>100</td>
<td>1364</td>
<td>881</td>
<td>280</td>
<td></td>
</tr>
<tr>
<td>DEX, MIX, and 0.5% anti-insulin serum</td>
<td>5</td>
<td>392</td>
<td>22.1</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>DEX, MIX, and 2% anti-insulin serum</td>
<td>0</td>
<td>266</td>
<td>1.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>DEX, MIX, and 5% anti-insulin serum</td>
<td>0</td>
<td>238</td>
<td>2.4</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>DEX, MIX, and 7.5% anti-insulin serum</td>
<td>0</td>
<td>232</td>
<td>2</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

### Table VII

**Reversal of anti-insulin serum-induced inhibition of fatty acid synthesis and the activities of stearoyl-CoA desaturase and fatty acid synthetase in 3T3-L1 adipocytes by the addition of insulin**

After confluence, culture dishes were either treated with dexamethasone (DEX) and methylisobutylxanthine (MIX) or with DEX, MIX, and 0.5% anti-insulin serum for 72 h. Dishes were then fed for 11 days with either normal medium or medium containing 0.5% anti-insulin serum supplemented with 1 µg and 10 µg of insulin/ml or no insulin. Cultures were pulsed for 1 h with [¹⁴C]acetate (10 µCi/dish).

<table>
<thead>
<tr>
<th>Treatment after confluence</th>
<th>Total fatty acids</th>
<th>Monoenoic acids</th>
<th>Stearoyl-CoA desaturase</th>
<th>Fatty acid synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[¹⁴C]Acetate incorporation into fatty acid</td>
<td>Enzyme activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>cpm × 10⁴</td>
<td>pmol/min/dish</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17.6</td>
<td>3.2</td>
<td>14</td>
<td>110</td>
</tr>
<tr>
<td>DEX and MIX</td>
<td>203</td>
<td>53</td>
<td>47</td>
<td>390</td>
</tr>
<tr>
<td>DEX, MIX, and 0.5% anti-insulin serum</td>
<td>14.1</td>
<td>3.2</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>DEX, MIX, and 0.5% anti-insulin serum + 10 µg of insulin/ml</td>
<td>805</td>
<td>227</td>
<td>330</td>
<td>4300</td>
</tr>
<tr>
<td>DEX, MIX, and 10 µg of insulin/ml</td>
<td>1515 489</td>
<td>340 4600</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>13.3</td>
<td>2.4</td>
<td>12</td>
<td>220</td>
</tr>
<tr>
<td>DEX and MIX</td>
<td>171</td>
<td>68</td>
<td>81</td>
<td>790</td>
</tr>
<tr>
<td>DEX, MIX, and 0.5% anti-insulin serum</td>
<td>16</td>
<td>4</td>
<td>10</td>
<td>98</td>
</tr>
<tr>
<td>DEX, MIX, and 0.5% anti-insulin serum + 1 µg of insulin/ml</td>
<td>350</td>
<td>168</td>
<td>244</td>
<td>2090</td>
</tr>
<tr>
<td>DEX, MIX, and 1 µg of insulin/ml</td>
<td>721 330</td>
<td>260 2160</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Insulin Regulation of Stearoyl-CoA Desaturase in 3T3-L1 Cells

The inhibition of de novo fatty acid synthesis in 3T3-L1 adipocytes by biotin deficiency is consistent with the results of other workers (13, 32). However, there is disagreement in literature as to whether biotin deficiency in 3T3-L1 cells affects phenotypic expression of lipid accumulation. Coleman et al. (13) and Freytag and Utrr (33) report that biotin deficiency has no effect on adipose conversion and lipid accumulation in 3T3-L1 cells whereas Kuri-Harcuch et al. (32) report that biotin deficiency, although not affecting morphologic differentiation, markedly inhibits lipid accumulation. Our present result is consistent with that of former workers (13, 33). The method of Kuri-Harcuch et al. (32), using di-alyzed serum, might be a more stringent method to obtain biotin deficiency, since it produced complete inhibition of [14C]acetate incorporation into fatty acids of 3T3-L1 cells. Lipoprotein lipase activity is greatly increased by insulin during adipose conversion (48, 49), and the activity is not affected by biotin deficiency (32). Thus, it appears that the extent of lipid accumulation in biotin-deficient 3T3-L1 adipocytes may depend on the amount of serum lipoproteins in the culture medium. Since stearoyl-CoA desaturase activity is not affected in biotin-deficient 3T3-L1 adipocytes, exogenous saturated fatty acids can be desaturated in order to maintain proper fluidity in the cellular phospholipids and triglycerides.

REFERENCES
Insulin Regulation of Stearoyl-CoA Desaturase in 3T3-L1 Cells