

# Phosphatidylcholine deficiency upregulates enzymes of triacylglycerol metabolism in CHO cells

J. Matías Caviglia,\* I. Nelva T. de Gómez Dumm,\* Rosalind A. Coleman,<sup>†</sup> and R. Ariel Igal<sup>1,\*</sup>

Instituto de Investigaciones Bioquímicas de La Plata,\* Universidad Nacional de La Plata, 1900-La Plata, Argentina; and Departments of Nutrition and Pediatrics,<sup>†</sup> University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

**Abstract** We studied the regulation of triacylglycerol (TAG) metabolism by phosphatidylcholine (PC) in CHO MT58 cells, which are deficient in PC synthesis because of a temperature-sensitive CTP:phosphocholine cytidyltransferase. At the permissive growth temperature (34°C), these cells contained 49% less TAG and 30% less PC than wild-type CHO K1 cells. Treatment with dipalmitoylphosphatidylcholine normalized both the PC and TAG levels. Despite low TAG levels, the incorporation of [<sup>14</sup>C]oleate into TAG was increased in CHO MT58 cells. The *in vitro de novo* synthesis of TAG and the activity of diacylglycerol acyltransferase were 90% and 34% higher, respectively. Two other key enzyme activities in TAG synthesis, acyl-CoA synthetase and mitochondrial glycerol-3-phosphate acyltransferase (GPAT), increased by 48% and 2-fold, respectively, and mitochondrial GPAT mRNA increased by ~4-fold. Additionally, TAG hydrolysis was accelerated in CHO MT58 cells, and *in vitro* lipolytic activity increased by 68%. These studies suggest that a homeostatic mechanism increases TAG synthesis and recycling in response to PC deficiency. TAG recycling produces diacylglycerol and fatty acids that can be substrates for *de novo* PC synthesis and for lysophosphatidylcholine (lysoPC) acylation. In CHO MT58 cells, in which *de novo* PC synthesis is blocked, lysoPC acylation with fatty acid originating from TAG may represent the main pathway for generating PC.—Caviglia, J. M., I. N. T. de Gómez Dumm, R. A. Coleman, and R. A. Igal. Phosphatidylcholine deficiency upregulates enzymes of triacylglycerol metabolism in CHO cells. *J. Lipid Res.* 2004. 45: 1500–1509.

**Supplementary key words** phosphatidylcholine metabolism • triacylglycerol synthesis • lipolysis • diacylglycerol acyltransferase • lipase

Phospholipids, which are molecules with both structural and potential signaling functions, and triacylglycerols (TAGs), which form the main energy store within cells, are metabolically interconnected by the exchange of common lipid intermediates. It has been observed that lipid intermediates of TAG and phospholipid metabolism, such

as diacylglycerol (DAG) and fatty acids, are exchanged in a recycling pathway that could be potentially relevant for membrane synthesis and lipid signal transduction (1, 2). Moreover, enzymes for both pathways are subject to common transcriptional regulation. For instance, the lipogenic transcriptional factor sterol regulatory element binding protein-1c has been shown to upregulate mitochondrial glycerol-3-phosphate acyltransferase (GPAT) and CTP:phosphocholine cytidyltransferase (CT), the critical enzymes for each pathway (3–5). However, little is known about the mechanism(s) of potential coregulation of TAG and phospholipid metabolism. Studies using permeabilized cells showed that TAG synthesis is an overflow system used when DAG accumulates as a result of limiting amounts of CDP-choline and that the rate of CDP-choline synthesis indirectly regulates TAG synthesis (6). However, we have recently provided evidence indicating that TAG synthetic enzymes, such as mitochondrial GPAT and diacylglycerol *O*-acyltransferase (DGAT), may indirectly regulate phospholipid synthesis by controlling the availability of lipid substrates (mainly fatty acids and DAG) for phospholipid formation (1, 2).

The relevance of TAG's roles in cell regulation goes beyond its well-studied function as the main cellular energy store. Recently, new roles for TAG metabolism in cell signaling and apoptosis have emerged. Accumulation of eicosapentaenoic acid-rich TAG in monocyte cells reduces proliferation (7). Movement of arachidonic acid between TAG and phospholipids has been observed during macrophage activation (8). It has also been shown that DAG and fatty acids released from plasma membrane phospholipids can be incorporated into TAG, suggesting a potential mechanism for attenuating lipid signals based on neutral

Abbreviations: ACS, acyl-CoA synthetase; CT, CTP:phosphocholine cytidyltransferase; DAG, diacylglycerol; DGAT, diacylglycerol *O*-acyltransferase; DPH, 1,6-diphenyl 1,3,5-hexatriene; DPPC, dipalmitoyl-phosphatidylcholine; GPAT, glycerol-3-phosphate acyltransferase; lysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; TAG, triacylglycerol.

<sup>1</sup> To whom correspondence should be addressed.  
e-mail: aigal@atlas.med.unlp.edu.ar

Manuscript received 25 February 2004 and in revised form 21 May 2004.

Published, JLR Papers in Press, June 1, 2004.  
DOI 10.1194/jlr.M400079.JLR200

This is an open access article under the [CC BY](https://creativecommons.org/licenses/by/4.0/) license.

Copyright © 2004 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at <http://www.jlr.org>

lipid synthesis (1, 2). Moreover, TAG metabolism seems to play a role in the modulation of the apoptotic program, although the data are still controversial. Thus, the association of a high TAG content in cells and the induction of programmed cell death through increased ceramide production has been described by Unger, Zhou, and Orci (9). On the other hand, Cnop et al. (10) postulated that pancreatic islet cells may accelerate the rate of TAG synthesis to remove excess free fatty acids that could promote apoptosis. This concept was further supported by the observation that TAG synthesis acts to protect against fatty acid-induced apoptosis in CHO cells (11).

In addition, TAG can contribute with both DAG and fatty acids to the synthesis of membrane phospholipids (12, 13). Compared with the supply from de novo synthesis, the contribution of TAG-derived lipids may be minor under normal culture conditions, but this mechanism could play an important role as an alternative route for membrane biogenesis. In this regard, a block in TAG-to-phospholipid recycling has been postulated to cause the abnormal accumulation of TAG in fibroblasts from patients with neutral lipid storage disease (13).

Maintaining a normal amount of membrane phospholipid, especially phosphatidylcholine (PC), is crucial for cell survival and replication. Under normal growth conditions, most PC is synthesized de novo from DAG and CDP-choline. The main regulated step in this pathway is the synthesis of CDP-choline by CT. The mutant CT-deficient strain CHO MT58 is a temperature-sensitive PC-deficient strain derived from CHO K1 cells (14, 15). At the restrictive temperature of 40°C, CT activity is blocked, leading to a decreased PC content, a slower growth rate, and, finally, apoptosis (14–16). At the permissive temperature (34°C), although the CT activity decreases by 20-fold, the cellular content of PC is reduced by only ~20% and the cell growth rate is almost normal (14, 17, 18). In CHO MT58 cells, the CT $\alpha$  gene has a point mutation within the catalytic domain and its protein expression is barely or not detectable, even at the permissive temperature (18, 19). Moreover, it was reported that CHO MT58 cells also express the isoform CT $\beta$  (20). The absence of CT activity in CHO MT58 grown at 40°C suggests that CT $\beta$  is also defective (20). In mouse macrophages, which also express CT $\alpha$  and CT $\beta$ 2 isoforms, when CT $\alpha$  is deleted by macrophage-specific knockout, the CT activity decreases to 10% of control values, CT $\beta$ 2 isoform expression is induced, and the cells are viable under normal growth conditions (21). Thus, in cells that normally express CT $\alpha$  and CT $\beta$ 2, the knockout of CT $\alpha$  does not eliminate all CT activity or compromise viability. Moreover, the fact that rescue of CHO MT58 cells by CT $\alpha$  overexpression requires an increase of CT activity over control values suggests that the mutation in CT $\alpha$  is not the only defect in this cell line (22).

When the de novo synthetic pathway cannot provide sufficient PC to meet the cell requirements, an alternative route for the synthesis of PC is used. CHO cells deprived of choline can take up lysophosphatidylcholine (lysoPC) from the culture medium to produce PC (23). In MT58

cells grown at 40°C, when the amount of PC synthesized de novo is deficient, PC is provided by acylating exogenous lysoPC with either exogenous or endogenously released fatty acids (24).

Because the metabolism of TAG and phospholipid is greatly interconnected, to determine how the regulation of TAG metabolism is influenced by PC levels, we studied TAG formation and recycling in CHO MT58 cells at the permissive temperature of 34°C, a condition in which these cells are viable despite a diminished content of PC. We present evidence demonstrating that mildly decreased levels of cellular PC promote a rapid cycle of TAG synthesis and lipolysis by activating in parallel the key enzymes of TAG synthesis acyl-CoA synthetase (ACS), mitochondrial GPAT, and DGAT as well as TAG lipase. These findings suggest that changes in cellular PC levels might modulate TAG metabolism to sustain normal levels of this phospholipid species.

## EXPERIMENTAL PROCEDURES

### Materials

CHO K1 cells were obtained from the American Type Culture Collection (Rockville, MD). CHO MT58 cells, a temperature-sensitive mutant deficient in CT isolated by Esko and Raetz (14), were a gift of Dr. Claudia Kent (University of Michigan). CHO cells stably transfected with CT $\alpha$  cDNA (CHO MT58/CT) were kindly donated by Dr. Dennis E. Vance (University of Alberta). Cell culture supplies, media, and other reagents were from Invitrogen Life Technologies (Grand Island, NY) and Corning Costar (Acton, MA). Ultrafiltered fetal bovine serum was from Nutrientes Celulares (Buenos Aires, Argentina). [ $^{14}\text{C}$ ]oleic acid and [ $\alpha$ - $^{32}\text{P}$ ]CTP were purchased from New England Nuclear (Boston, MA). BSA and Protease Inhibitor Cocktail for General Use were from Sigma (St. Louis, MO). Pure lipid standards were from Doosan Serdary (Yongin, Korea). 1,6-Diphenyl 1,3,5-hexatriene (DPH) was purchased from Aldrich (Milwaukee, WI). Silica gel 60 chromatography plates were from Merck (Darmstadt, Germany). Trizol was from Invitrogen (Carlsbad, CA). Hybond-N $^{+}$  membranes were from Amersham Biosciences (Piscataway, NJ).

### Cell culture and radiolabeling

Cells were routinely grown at 34°C in 100 mm petri dishes in Ham's F12 medium with 10% heat-inactivated FBS plus 100 U/ml penicillin, 100  $\mu\text{g}/\text{ml}$  streptomycin, and 5%  $\text{CO}_2$ . CT $\alpha$ -transfected CHO MT58 cells were cultured in the same medium plus 300  $\mu\text{g}/\text{ml}$  G418. For some experiments, liposomes of dipalmitoyl-phosphatidylcholine (DPPC) in phosphate-buffered saline were prepared by sonicating the lipid suspension twice for 5 min on ice according to the protocol of Esko, Nishijima, and Raetz (24). For the labeling experiments, cells were incubated with 0.25  $\mu\text{Ci}$  of [ $^{14}\text{C}$ ]oleate plus 100  $\mu\text{M}$  unlabeled oleate in medium supplemented with 0.5% BSA. At the end of each incubation, the medium was aspirated, the monolayers were washed twice with ice-cold phosphate-buffered saline plus 0.1% BSA, and total lipids were extracted and analyzed as described below.

### Preparation of the subcellular fraction and enzyme assays

CHO cells were grown in 100 mm dishes to 80–90% confluence. For DGAT and TAG lipase activity assays, cells were trypsinized, washed twice with ice-cold PBS, resuspended in 10

mM Tris-HCl buffer, pH 7.4, with Protease Inhibitor Cocktail, and homogenized by sonication on ice for 10 s at 50% output. For ACS and GPAT activity assays, cells were resuspended in 10 mM Tris, pH 7.4, 250 mM sucrose, 1 mM EDTA, and 1 mM dithiothreitol, and homogenates were obtained by 10 up-and-down strokes in a Teflon-glass homogenizer. The total particulate fraction was prepared by centrifuging the homogenates for 1 h at 100,000 *g* at 4°C. Homogenates and total particulate preparations were stored in aliquots at -70°C until use.

De novo lipid synthesis was measured according to the protocol of Lloyd-Davies and Brindley (25). Total particulate fractions (500 µg of protein) were incubated with 128 µM [<sup>14</sup>C]oleate, 20 mM α-glycerol phosphate, 0.2 µM CoA, 2.8 mM *N*-acetyl-L-cysteine, and 1.1 mM MgCl<sub>2</sub> in 50 mM Tris, pH 7.4. The incubation was carried out at 37°C for 60 min, then lipids were extracted, separated, and analyzed as described below.

DGAT activity was determined using 100 and 200 µg of total particulate protein, 200 µM DAG in acetone, and 30 µM [<sup>3</sup>H]palmitoyl-CoA in buffer (175 mM Tris, pH 8, 8 mM MgCl<sub>2</sub>, and 1 mg/ml BSA) at 37°C for 30 min according to the procedure of Coleman (26). ACS activity was assayed with 2.5–10 µg of homogenate, 50 µM [<sup>14</sup>C]palmitate, 10 mM ATP, and 0.25 mM CoA in 175 mM Tris, pH 7.4, 8 mM MgCl<sub>2</sub> at 37°C for 5 min (27). GPAT activity assays contained 3.5–15 µg of total particulate protein, 0.8 mM [<sup>3</sup>H]glycerol-3-phosphate, 82.5 µM palmitoyl-CoA, 75 mM Tris, pH 7.4, 4 mM MgCl<sub>2</sub>, 2 mg/ml BSA, 8 mM NaF, and 1 mM DTT and were incubated at 37°C for 10 min (28). The fractions were preincubated for 15 min on ice with 1 mM *N*-ethylmaleimide for measuring mitochondrial GPAT. Microsomal GPAT activity was calculated by subtracting mitochondrial from total GPAT activity, which was measured without *N*-ethylmaleimide. TAG lipase activity was assayed using 200 µg of homogenate protein and 1 µM tri[<sup>14</sup>C]olein in 175 µM Tris-HCl, pH 7.8, with 1 mg/ml BSA. The incubation was performed at 37°C for 60 min. Lipids were then extracted, and free [<sup>14</sup>C]oleic acid was separated by TLC and counted in a liquid scintillation counter (see below).

### Lipid extraction and analysis

Cell monolayers were washed twice and scraped from the dishes with two additions of 1 ml of methanol and 0.5 ml of distilled water. Finally, 1 ml of chloroform was added, and lipid extractions were completed as described by Bligh and Dyer (29). Lipids were separated by TLC using silica gel 60 plates. Neutral lipids were resolved using hexane-ethyl ether-acetic acid (80:20:2, v/v), and phospholipids were separated with chloroform-methanol-acetic acid-water (50:37.5:3.5:2, v/v). To quantify the lipid species, known amounts of lipid standards were run on each plate and 100 µM DPH was added to the solvent system (30). The lipid spots were visualized by fluorescence emission of DPH and quantified with a Kodak Digital Science DS 120 image system. <sup>14</sup>C-labeled lipids were scraped from the plates and quantified in a liquid scintillation counter.

### Northern blot analysis

Total RNA was isolated from preconfluent cells grown at 34°C using Trizol reagent according to the manufacturer's instructions. The RNA (20 µg) was separated on a 1% agarose gel containing 2.2 M formaldehyde and transferred to Hybond-N<sup>+</sup> nylon membranes. The following probes, a 0.8 kb *Eco*RI fragment from rat mitochondrial GPAT and a 0.8 kb *Pvu*II fragment from mouse DGAT1, were labeled with [α-<sup>32</sup>P]CTP by random priming and used to perform Northern blot hybridization (31). The blots were exposed to a Phosphor screen, and the signals were quantified with a Storm 840 system (Molecular Dynamics). A

GAPDH probe was used as a loading control for normalizing the signals.

### Other methods

Protein content was measured as described by Lowry et al. (32). Total DNA content was measured according to the procedure of Labarca and Paigen (33). [<sup>3</sup>H]palmitoyl-CoA and [<sup>3</sup>H]glycerol-3-phosphate were synthesized enzymatically (34, 35). Data were tested for statistical significance using Student's *t*-test or one-way ANOVA.

## RESULTS

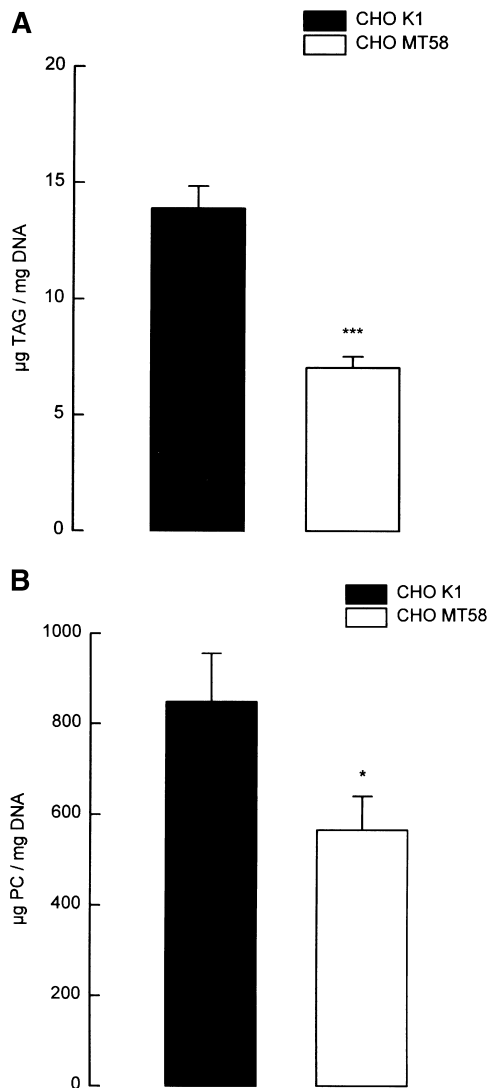
### Levels of PC and TAG in wild-type and CT-deficient CHO MT58 cells

Although phospholipids and TAG have exclusive functions, these lipids are potential subjects for joint regulation because they share common substrates and metabolic routes. To investigate the extent of common regulation between PC and TAG metabolism, we used a cell model for PC deficiency, the CT-deficient CHO MT58 cells. At 34°C, these cells contain levels of PC lower than control cells; however, they are viable and grow at an almost normal rate (14, 22), allowing studies during extended time courses and avoiding the confounding effects of apoptosis on lipid metabolism.

Initially, we analyzed the pool size of TAG and PC in wild-type and CT-deficient CHO cells. Surprisingly, our results showed that the CHO MT58 cells grown at 34°C reduced their TAG mass by 49%, whereas they exhibited a 33% decrease in PC content compared with wild-type CHO K1 cells (Fig. 1A, B). This indicates that the reduced de novo synthesis of PC markedly affected TAG metabolism.

Wild-type CHO K1 cells expand their TAG pool when they are incubated with 100 µM oleate (1, 36). To determine whether CHO MT58 cells were able to increase their TAG content when cultured in the presence of fatty acids, we incubated CHO K1 and CHO MT58 cells with 100 µM oleate for 4, 8, and 24 h. CHO MT58 cells were able to increase their TAG content, but the amount of TAG remained 38–60% lower than in CHO K1 cells at all incubation times (Fig. 2A), indicating that TAG deficiency in the CT-mutant cells was not attributable to substrate availability. Because oleic acid is a well-known activator of CT activity (37), we also determined the levels of cellular PC in control and CT-mutant CHO cells (Fig. 2B). Treatment with oleic acid for 24 h slightly increased the PC content in both cell groups, but the levels of PC in the CHO MT58 cells remained lower than those in the wild-type cell strain during the treatment period.

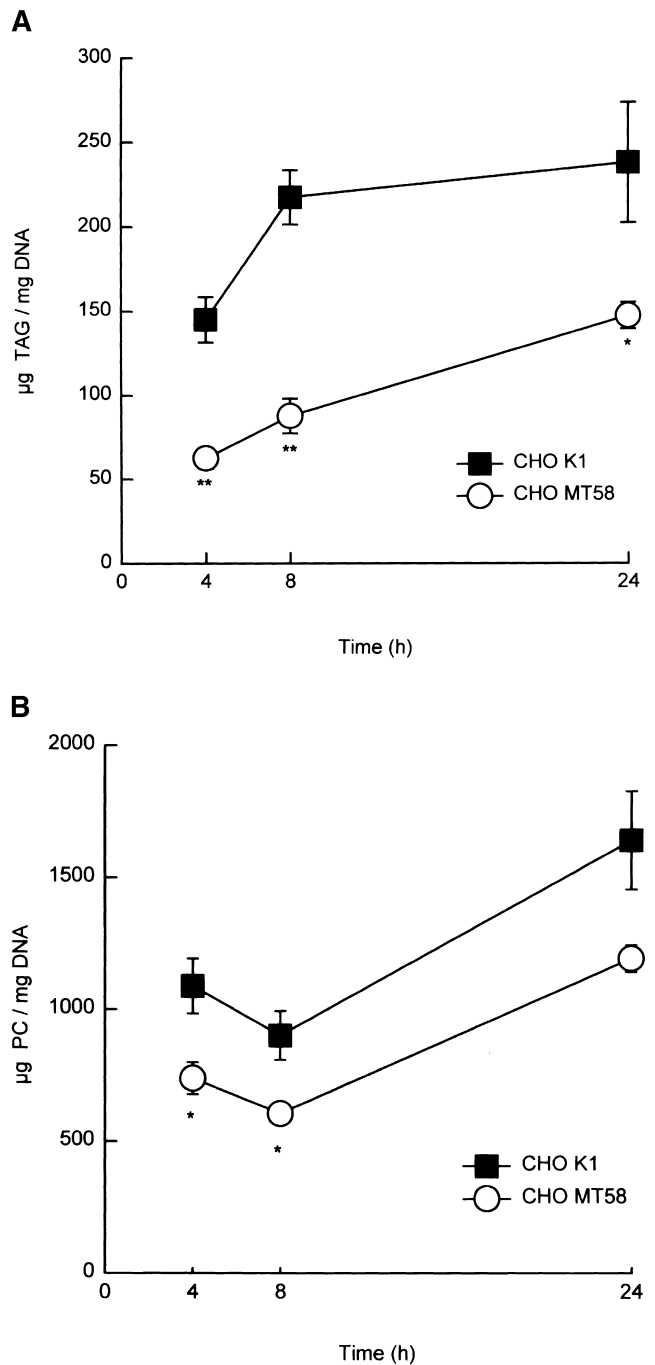
Taking these observations into account, we next asked whether the diminished TAG pool found in CHO MT58 cells was directly linked to the subnormal levels of cell PC. Hence, we examined whether normalization of PC levels would restore TAG content to normal values. PC-deficient cells are able to restore their PC content by incorporating PC supplied as liposomes in the culture medium (23, 24). CHO MT58 cells were grown for 72 h in medium contain-



**Fig. 1.** Triacylglycerol (TAG) and phosphatidylcholine (PC) contents are decreased in CHO MT58 cells. CHO K1 and CHO MT58 cells were cultured in standard medium; their lipids were extracted and separated by TLC. TAG (A) and PC (B) amounts were quantitated by 1,6-diphenyl 1,3,5-hexatriene (DPH) fluorescence. The experiment was repeated three times with similar results. Data represent means  $\pm$  SE of quadruplicate (for TAG) and triplicate (for PC) determinations. \*  $P < 0.05$  and \*\*\*  $P < 0.001$  by Student's *t*-test.

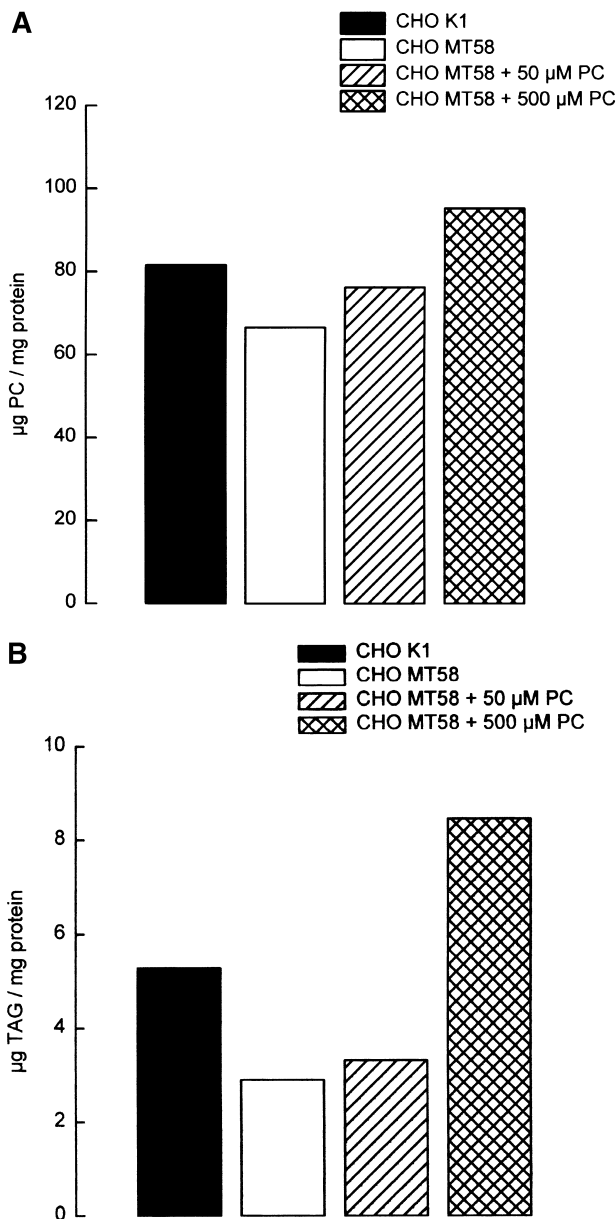
ing either 50 or 500  $\mu\text{M}$  DPPC liposomes. CHO MT58 cells treated with 50  $\mu\text{M}$  DPPC increased their PC content, but only the addition of 500  $\mu\text{M}$  DPPC fully restored the levels of cell PC to values found in wild-type cells (**Fig. 3A**). Although 50  $\mu\text{M}$  DPPC nearly normalized the decreased PC values in the CT-deficient cells, it did not increase the low TAG mass (**Fig. 3B**). Only when the addition of 500  $\mu\text{M}$  DPPC restored the PC mass to levels higher than those found in control cells did the TAG pool expand to levels higher than in nontreated CHO MT58 cells (2.9-fold) or wild-type cells (60%).

Because exogenous PC, although present in high amounts, was capable of restoring the TAG pool, we reasoned that



**Fig. 2.** TAG content is low in CHO MT58 cells treated with 100  $\mu\text{M}$  oleate. Cells were treated for 4, 8, and 24 h with 100  $\mu\text{M}$  oleate. Lipids were extracted and separated by TLC. TAG (A) and PC (B) content was measured by fluorescence emission of DPH. Data represent means  $\pm$  SE of triplicate determinations. \*  $P < 0.05$  and \*\*  $P < 0.01$  compared with control values (CHO K1).

the diminished mass of TAG found in CHO MT58 cells was related to their low PC content. Thus, we asked whether CHO MT58 cells stably transfected with CT $\alpha$  cDNA, which restores PC content to normal at the permissive temperature (22), could also normalize the cell TAG mass. Although expression of CT $\alpha$  in CHO MT58 cells normalized PC levels (CHO K1,  $53.72 \pm 6.82$   $\mu\text{g}/\text{mg}$  pro-

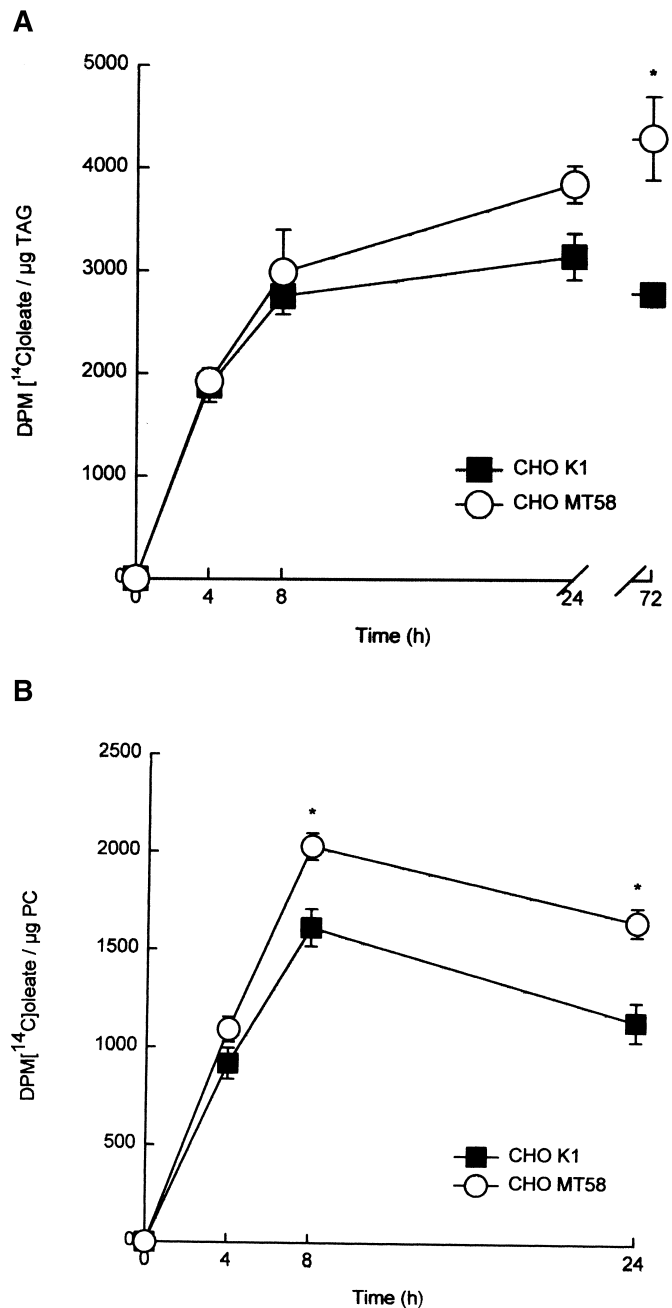


**Fig. 3.** Dipalmitoyl-phosphatidylcholine (DPPC) liposomes restore TAG content in CTP:phosphocholine cytidyltransferase (CT)-deficient cells. CHO MT58 cells were incubated in medium that contained either 50 or 500  $\mu\text{M}$  DPPC liposomes for 72 h. CHO K1 and CHO MT58 cells grown in regular culture medium were used as controls. The medium was replaced every day. Lipids were extracted and PC (A) and TAG (B) mass was quantified by TLC and DPH fluorescence emission. Values represent averages of duplicate samples, which varied less than 5%.

tein; CHO MT58/CT,  $54.38 \pm 0.4 \mu\text{g}/\text{mg}$  protein), it did not restore TAG content to wild-type levels even when 100  $\mu\text{M}$  oleic acid was added to the medium (data not shown). Thus, the endogenous production of normal amounts of PC by CT $\alpha$  was not sufficient to restore the TAG pool to values found in wild-type cells.

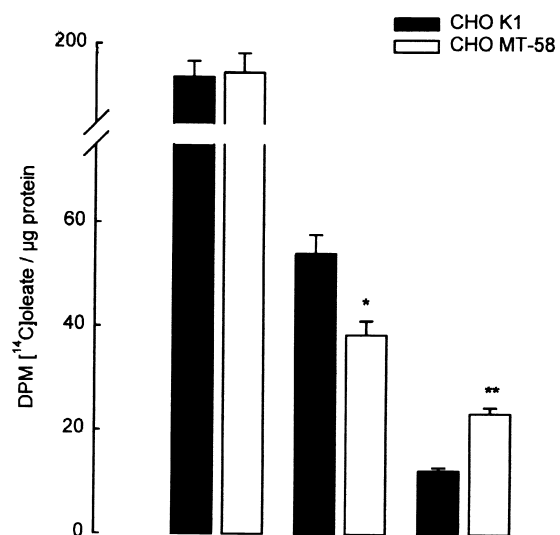
#### Synthesis of PC and TAG in CHO MT58 cells

To determine whether the low TAG mass resulted from a decrease in TAG synthesis in the CHO MT58 cells, we



**Fig. 4.** The incorporation of [ $^{14}\text{C}$ ]oleate into TAG and PC is increased in CHO MT58 cells. Preconfluent cells were incubated with 100  $\mu\text{M}$  [ $^{14}\text{C}$ ]oleate for 4, 8, 24, and 72 h. Lipids were extracted and radiolabel incorporation and lipid mass were determined. The specific activities of [ $^{14}\text{C}$ ]TAG (A) and [ $^{14}\text{C}$ ]PC (B) were calculated and plotted against time. Values represent means  $\pm$  SE of triplicate determinations. \*  $P < 0.05$ . DPM, disintegrations per minute.

traced the incorporation of [ $^{14}\text{C}$ ]oleate into the TAG pool. Because the pool size of TAG differed in CT-deficient and wild-type cells, we used a more precise measure of the TAG synthetic rate by examining changes in the pool-specific activities. CHO MT58 and control cells were incubated with 100  $\mu\text{M}$  [ $^{14}\text{C}$ ]oleate for as long as 72 h. The incorporation of [ $^{14}\text{C}$ ]oleate into TAG showed similar levels for both cell types for up to 24 h (Fig. 4A). How-



**Fig. 5.** De novo lipid synthesis in cell membranes. Membrane pellets from CHO K1 and CHO MT58 cells were incubated with  $\alpha$ -glycerol-3-phosphate, [ $^{14}$ C]oleate, and cofactors for 60 min as described in Experimental Procedures. Lipids were extracted and separated by TLC, and incorporation of radiolabel was quantified. Values represent means  $\pm$  SE of three determinations. \*  $P < 0.05$  and \*\*  $P < 0.01$ .

ever, at 72 h of incubation, the specific activity of the TAG pool in CHO MT58 cells was 55% higher. The increased [ $^{14}$ C]TAG specific activity found in CHO MT58 cells would indicate an accelerated turnover of their TAG stores. The specific activity of the [ $^{14}$ C]PC pool was higher by 26% and 45% in MT58 cells at 8 and 24 h, respectively, indicating rapid fatty acid recycling in this phospholipid pool (Fig. 4B). These differences are not attributable to an increased uptake of oleate, because the total uptake was actually decreased in CHO MT58 cells (data not shown).

To confirm that the presence of a more highly labeled TAG pool was derived from enhanced TAG synthesis, we determined the de novo formation of neutral lipids in total cell membranes isolated from CHO K1 and CHO MT58 cells (Fig. 5). The incorporation of [ $^{14}$ C]oleate into TAG was increased by 90% in CHO MT58 cells compared with the wild-type strain. This higher production of TAG was accompanied by a 29% decrease in DAG labeling. The other intermediate in the route of TAG synthesis

from  $\alpha$ -glycerol phosphate, phosphatidic acid, showed no changes. Lysophosphatidic acid was not detected in the cell-free assay.

The opposite changes in the formation of DAG and TAG, which are substrate and product, respectively, of the enzyme DGAT, suggested that this enzymatic activity might be increased in CHO MT58 cells. Indeed, we observed that CHO MT58 cells exhibited a 34% increase in DGAT activity compared with control cells (Table 1).

Additionally, we measured two other enzymes of the glycerolipid synthetic pathway that are related to TAG synthesis. ACS activity was 48% and 50% higher in CHO MT58 and CHO MT58/CT cells, respectively, compared with the CHO K1 strain. Mitochondrial GPAT activity was 2-fold higher in CHO MT58 and CHO MT58/CT cells compared with CHO K1 cells (Table 1). Microsomal GPAT activity did not change.

Mitochondrial GPAT, DGAT1, and DGAT2 are regulated transcriptionally (3, 38). To determine whether the changes in enzyme activity were attributable to gene expression, Northern blot analysis of CHO K1, CHO MT58, and CHO MT58/CT cells was performed with mitochondrial GPAT and DGAT1 probes. Mitochondrial GPAT mRNA showed an average 3.5-fold increase in CHO MT58 cells; no changes were detected for DGAT1 (Fig. 6).

Taken as a whole, these results are consistent with an increased rate of TAG synthesis that cannot account for the lower TAG mass in CHO MT58 cells at 34°C. Additionally, these observations suggest that PC deficiency may increase the activity and expression of enzymes involved in the synthesis of acylglycerol molecules.

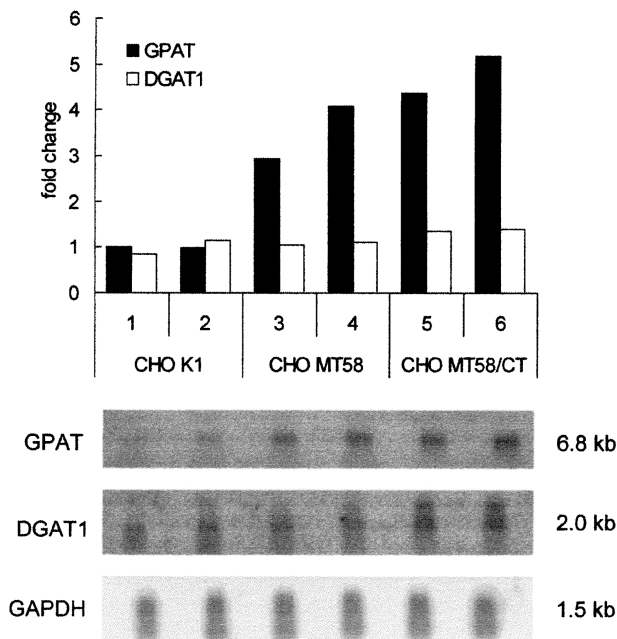
#### Rate of lipolysis in CHO MT58 cells

Cellular TAG content results from a balance between synthesis and hydrolysis. Because CHO MT58 cells contained less TAG despite an increase in TAG synthesis, we examined the role of TAG lipolysis. Preconfluent cells were incubated for 72 h with 100  $\mu$ M [ $^{14}$ C]oleate to label the TAG pool. Cells were then chased in medium without added oleate for up to 96 h, and the [ $^{14}$ C]TAG specific activity was determined (Fig. 7A). In wild-type CHO cells, the radiolabeled TAG decreased by 4, 11, 23, and 49% at 6, 24, 48, and 96 h, respectively, corresponding to a steady mobilization rate of 12% every 24 h. In contrast, at these time points the labeled TAG pool in CHO MT58 cells was reduced by 34, 59, 55, and 64%, respectively, indicating that TAG hydrolysis was more rapid in the CT-deficient

TABLE 1. Glycerolipid synthetic enzyme activities in CHO K1, CHO MT58, and CHO MT58/CT cells

Cell Line	Diacylglycerol Acyltransferase	Acyl-CoA Synthetase	Mitochondrial GPAT	Microsomal GPAT
	<i>nmol/min/mg protein</i>			
CHO K1	0.083 $\pm$ 0.006a	4.75 $\pm$ 0.40a	0.18 $\pm$ 0.01a	1.47 $\pm$ 0.19a
CHO MT58	0.112 $\pm$ 0.003b	7.02 $\pm$ 0.16b	0.37 $\pm$ 0.01b	1.48 $\pm$ 0.16a
CHO MT58/CT	N.D.	7.09 $\pm$ 0.036b	0.38 $\pm$ 0.01b	1.90 $\pm$ 0.12a

Diacylglycerol acyltransferase, acyl-CoA synthetase, mitochondrial glycerol-3-phosphate acyltransferase (GPAT), and microsomal GPAT activities were measured as described in Experimental Procedures. Data are averages of triplicate determinations  $\pm$  SE. Values not bearing the same letter are significantly different at  $P < 0.05$ . CT, CTP:phosphocholine cytidyltransferase; N.D., not determined.



**Fig. 6.** Expression of mitochondrial glycerol-3-phosphate acyltransferase (GPAT) mRNA is increased in CHO MT58 and CHO MT58/CT cells. Total RNA (20  $\mu$ g) from cells cultivated at 34°C was subjected to Northern blot hybridization with labeled probes against rat mitochondrial GPAT, mouse diacylglycerol *O*-acyltransferase-1 (DGAT1), and GAPDH and quantified with a PhosphorImager. The ratios of mitochondrial GPAT/GAPDH and DGAT1/GAPDH were calculated, and the fold changes relative to the average for control CHO K1 cells were plotted.

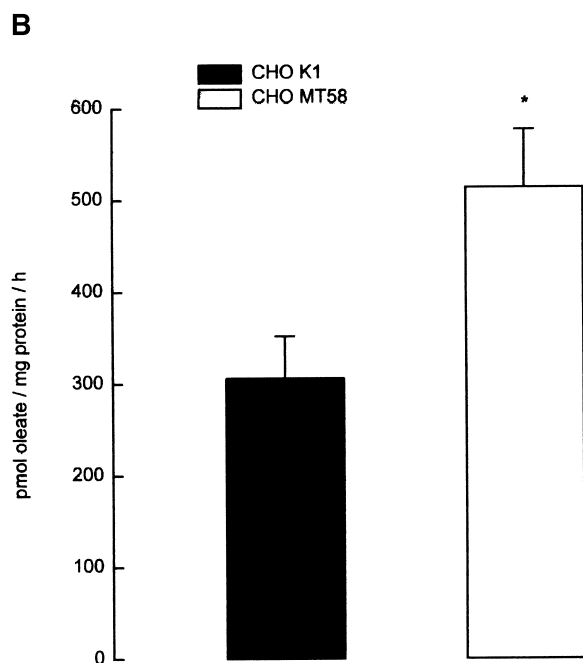
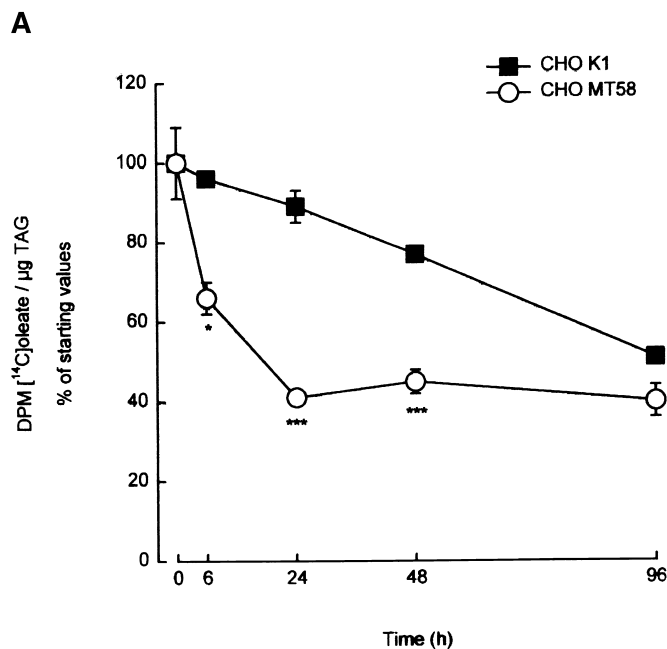
CHO MT58 cells. The CHO MT58 cells released the major portion of their  $^{14}$ C label by 24 h; little label was lost thereafter, probably because released fatty acids recycled back into TAG.

To further investigate TAG hydrolysis, the *in vitro* TAG lipase activity was measured in cell homogenates. As shown in Fig. 7B, the lipase activity was increased by 68% in CHO MT58 cells compared with control CHO K1 cells. These results suggest that the reduced TAG pool found in CHO MT58 cells is caused by a greater lipolytic rate.

## DISCUSSION

An emerging body of data indicates that in mammalian cells in which TAG is not stored for energetic purposes, TAG metabolism plays a critical role in functions such as signaling and membrane lipid synthesis. Moreover, excess TAG in nonadipose cells such as pancreatic  $\beta$ -cells and cardiac cells is thought to be a marker of or to be responsible for the onset of diseases such as type 2 diabetes mellitus and heart dysfunction (39, 40). Nevertheless, the regulation of TAG synthesis and recycling in nonadipose cells is still not well understood.

Although in several cell types it has been shown that TAG synthesis operates as an overflow system when substrate concentrations for phospholipid metabolism are limiting (6, 41, 42), recent work from our laboratory sug-



**Fig. 7.** CHO MT58 cells show a higher lipolytic rate and *in vitro* TAG lipase activity. A: To measure the TAG lipolytic rate, the cellular TAG pool was labeled with 100  $\mu$ M [ $^{14}$ C]oleate for 72 h and then chased for up to 96 h in fresh medium lacking oleate. Lipids were extracted and the mass and amount of [ $^{14}$ C]oleate incorporated into TAG were quantified. The specific radioactivity expressed as a percentage of the starting values was plotted against time. The values at time 0 were 2,787 dpm/ $\mu$ g TAG (CHO K1) and 4,310 dpm/ $\mu$ g TAG (CHO MT58). Values are means  $\pm$  SE of three determinations. \*  $P < 0.05$  and \*\*\*  $P < 0.001$ . B: Cells grown in 100 mm petri dishes to near confluence were harvested and homogenized by sonication. Lipase activity was assayed in the homogenate fractions as described in Experimental Procedures and expressed as picomoles of oleate produced per milligram of protein per hour. Values are means  $\pm$  SE of four determinations. \*  $P < 0.05$ .

gests that DGAT, the terminal enzyme in TAG synthesis, aids in the partitioning of the lipid substrates DAG and fatty acids between TAG and phospholipid synthetic pathways (2). However, the question of whether TAG synthesis and recycling depends on phospholipid homeostasis in growing cells has not yet been examined.

The data presented here indicate that the pool size of cellular TAG depends on the levels of the main membrane phospholipid, PC, and is likely a part of an alternative mechanism for supporting membrane lipid synthesis. The dependence of TAG on PC content is founded on two main observations: *a*) growing CT-deficient CHO cells, which contain ~30% lower levels of PC at the permissive temperature, also contain a TAG pool that is ~50% decreased; and *b*) the lower TAG content in MT58 cells increases when cellular PC levels are fully normalized by adding exogenous PC.

Additionally, the fact that low TAG levels are present in CT $\alpha$ -expressing MT58 cells, which contain normal levels of PC synthesized endogenously, strongly suggests that TAG metabolism depends not only on PC mass. Full normalization of TAG may also depend on complete restoration of PC metabolism regarding intracellular pools and molecular species as well. In this regard, two CT genes encoding four different isoforms have been identified (43). These CT isoforms are coexpressed in several tissues and cell lines, and they have different subcellular locations, tissue expression distribution, and regulatory sequences (20, 43, 44). These observations suggest that the CT isoforms may have different functions (44). Although overexpression of any of the isoforms (CT $\alpha$ , CT $\beta_1$ , CT $\beta_2$ , or CT $\beta_3$ ) supports the growth of CHO MT58 cells at 40°C (18, 20, 22, 43), not every isoform will necessarily restore all alterations in CHO MT58 cells. We hypothesize that cells might need to fully restore the PC homeostasis to attain a normal TAG pool. An alternate possibility is that cells must contain a PC pool with a specific fatty acid composition to normalize the cellular TAG content. Indeed, it has been observed that growth in CHO MT58 cells can be rescued at the nonpermissive temperature by adding exogenous desaturated PC but not unsaturated PC (24).

The presence of low TAG content in MT58 cells is explained by the finding of rapid TAG recycling, in which the rates of both TAG synthesis and degradation were accelerated. On the synthetic side, the activities of key enzymes of TAG synthesis, ACS, mitochondrial GPAT, and DGAT, were increased. In CHO MT58 cells, ACS and mitochondrial GPAT activities were significantly increased with respect to controls (~50% and 2-fold, respectively). Although these enzymes are not unique for TAG synthesis, both have been proposed to channel fatty acids toward TAG synthesis (36, 45, 46). In addition, the changes in mitochondrial GPAT activity are caused by induction of mRNA expression, strongly suggesting that gene expression of lipogenic enzymes depends on PC homeostasis. Furthermore, the activity of DGAT, which catalyzes the only committed step in TAG synthesis and has been proposed to play a regulatory role, is increased by ~35% in CHO MT58 cells compared with CHO K1 cells. DGAT1

mRNA showed no changes between cell types, suggesting that the increased DGAT activity may be the product of another isoform such as DGAT2 and/or posttranscriptional upregulation.

In addition to the upregulation of lipogenic enzyme activities, another mechanism that may contribute to the increased TAG synthesis is the diversion of lipid intermediates from PC synthesis toward TAG. In this regard, it has been shown that when CHO MT58 cells are grown at 40°C and PC synthesis is blocked, TAG synthesis increases by rechanneling lipid substrates for PC formation toward TAG (41, 42). In CHO MT58 cells grown at the permissive temperature, the depression of de novo synthesis of PC is not as severe as at 40°C (18), but it may be low enough to divert lipid substrates (DAG and fatty acids) not used for phospholipid synthesis toward TAG synthesis. Our observations of a high TAG synthetic rate in cells when the amount of CDP-choline is limited are also in agreement with studies performed in permeabilized cells incubated with low levels of CDP-choline (47). In this cell model of CDP-choline depletion, it was shown that DAG molecules accumulate and that DGAT exhibits its maximal activity, which together allow a fully activated TAG formation.

On the other hand, the discrepancy between the high rate of TAG formation and the low levels of TAG is elucidated by the data provided by the *in vivo* and *in vitro* lipolysis experiments. The presence of a smaller TAG store in growing CHO MT58 cells might be explained by rapid lipolysis that overcomes the high TAG synthetic rate. The lipolytic rate was more accelerated in CHO MT58 cells than in control cells. The CT-deficient cells depleted ~50% of their TAG pool by 24 h before reaching a plateau, whereas it took the control CHO K1 cells 96 h to reduce their TAG pool to a similar value. The rapid lipolytic rate in CT-deficient cells was confirmed by an *in vitro* TAG lipase activity that was ~70% higher than in normal cells.

To date, there are no available data indicating that PC content may affect the rate of TAG metabolism. We may hypothesize that one reason for the rapid recycling of depot TAG in normally growing PC-deficient CHO cells may lie in an alteration in the structure of the lipid droplets. TAG and CE are stored in cytoplasmic structures in which they form a core of neutral lipids surrounded by a surface layer of phospholipid and amphipathic proteins (48). Perilipin, one of the proteins that surrounds lipid droplets in adipocytes, protects the TAG against lipase activity (49); adipose differentiation-related protein, the comparable protein in CHO cells, is thought to have a similar function (50). The PC deficiency in CHO MT58 cells may change the constitution of the phospholipid monolayer surrounding the lipid droplets and make TAG more accessible to lipases. Hence, in a condition in which the cell cannot maintain normal levels of PC to sustain membrane integrity, less PC might be available for lipid droplet formation, making the TAG stores more accessible to lipolysis. This effect has been observed in choline-starved hepatocytes. With choline depletion, hepatocytes produce less PC, and the resulting VLDL, whose protein and lipid are structurally disposed in a manner similar to that of lipid droplets,



exhibits an altered structure that leads to an increased intracellular degradation and less VLDL secretion (51).

Although structural changes in lipid droplets caused by a lack of sufficient PC could hypothetically promote rapid TAG recycling, no evidence supports this type of effect. It is more likely that the higher rate of TAG synthesis and mobilization in a state of mild PC deficiency is part of a homeostatic mechanism to provide normal levels of PC for membrane biogenesis in growing cells. Mammalian cells can use exogenous PC for membrane biogenesis during choline starvation or when the endogenous production of PC is altered (23, 24). After incorporation into cells, exogenous PC can make up to 50% of the total cellular PC. However, an important activity of fatty acid remodeling in phospholipids may be obligatory, because lysoPC can restore the growth of CHO MT58 cells but ether-linked PC cannot, indicating that lysoPC acyltransferase might generate an important amount of PC (24). In our experiments, in parallel with TAG recycling, we observed a higher rate of incorporation of exogenous oleate into the cellular PC pool of MT58 cells, which suggests a high rate of fatty acid remodeling. Because the de novo synthesis of PC is depressed in these cells, the formation of new PC may occur via the acylation of lysoPC by fatty acids, potentially originating from TAG lipolysis. Nevertheless, the contribution of fatty acids from serum cannot be ruled out because the culture medium included  $\sim 10 \mu\text{M}$  free fatty acids from serum (data not shown). At  $40^\circ\text{C}$ , neither de novo synthesis nor reacylation of lysoPC enabled cells to sustain a sufficient rate of PC formation, and the cell death program was triggered. In this terminal condition, providing lipid substrates from TAG to support phospholipid synthesis might no longer be necessary. Lipid substrates initially destined for PC synthesis are diverted toward TAG synthesis, overcoming its mobilization and resulting in net TAG accumulation (41, 42).

In summary, we postulate that PC metabolism affects TAG synthesis and mobilization. This mechanism of TAG-to-phospholipid recycling may operate to provide lipid substrates for phospholipid formation when its de novo synthesis is blocked. **FIG**

The present work was supported by grants from the National Institutes of Health (TW-00891 to R.A.I. and R.A.C. and DK-59935 to R.A.C.), the Agencia Nacional de Promoción Científica y Tecnológica (PICT 06-03823 to R.A.I.), and the Fundación Antorchas (A-13462 to R.A.I.). R.A.I. and I.N.T.d.G.D. are members of the Scientific Research Career from the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Argentina. J.M.C. is a Doctoral Fellow from CONICET, Argentina.

## REFERENCES

- Igal, R. A., J. M. Caviglia, I. N. de Gomez Dumm, and R. A. Coleman. 2001. Diacylglycerol generated in CHO cell plasma membrane by phospholipase C is used for triacylglycerol synthesis. *J. Lipid Res.* **42**: 88–95.
- Bagnato, C., and R. A. Igal. 2003. Overexpression of diacylglycerol acyltransferase-1 reduces phospholipid synthesis, proliferation, and invasiveness in simian virus 40-transformed human lung fibroblasts. *J. Biol. Chem.* **278**: 52203–52211.
- Ericsson, J., S. M. Jackson, J. B. Kim, B. M. Spiegelman, and P. A. Edwards. 1997. Identification of glycerol-3-phosphate acyltransferase as an adipocyte determination and differentiation factor 1- and sterol regulatory element-binding protein-responsive gene. *J. Biol. Chem.* **272**: 7298–7305.
- Kast, H. R., C. M. Nguyen, A. M. Anisfeld, J. Ericsson, and P. A. Edwards. 2001. CTP:phosphocholine cytidyltransferase, a new sterol- and SREBP-responsive gene. *J. Lipid Res.* **42**: 1266–1272.
- Lagace, T. A., M. K. Storey, and N. D. Ridgway. 2000. Regulation of phosphatidylcholine metabolism in Chinese hamster ovary cells by the sterol regulatory element-binding protein (SREBP)/SREBP cleavage-activating protein pathway. *J. Biol. Chem.* **275**: 14367–14374.
- Stals, H. K., G. P. Mannaerts, and P. E. Declercq. 1992. Factors influencing triacylglycerol synthesis in permeabilized rat hepatocytes. *Biochem. J.* **283**: 719–725.
- Finstad, H. S., C. A. Drevon, M. A. Kulseth, A. V. Synstad, E. Knudsen, and S. O. Kolset. 1998. Cell proliferation, apoptosis and accumulation of lipid droplets in U937-1 cells incubated with eicosapentaenoic acid. *Biochem. J.* **336**: 451–459.
- Triggiani, M., A. Oriente, and G. Marone. 1994. Differential roles for triglyceride and phospholipid pools of arachidonic acid in human lung macrophages. *J. Immunol.* **152**: 1394–1403.
- Unger, R. H., Y. T. Zhou, and L. Orci. 1999. Regulation of fatty acid homeostasis in cells: novel role of leptin. *Proc. Natl. Acad. Sci. USA.* **96**: 2327–2332.
- Cnop, M., J. C. Hannaert, A. Hoorens, D. L. Eizirik, and D. G. Pipeleers. 2001. Inverse relationship between cytotoxicity of free fatty acids in pancreatic islet cells and cellular triglyceride accumulation. *Diabetes.* **50**: 1771–1777.
- Listenberger, L. L., X. Han, S. E. Lewis, S. Cases, R. V. Farese, Jr., D. S. Ory, and J. E. Schaffer. 2003. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc. Natl. Acad. Sci. USA.* **100**: 3077–3082.
- Cook, H. W., and M. W. Spence. 1985. Triacylglycerol as a precursor in phospholipid biosynthesis in cultured neuroblastoma cells: studies with labeled glucose, fatty acid, and triacylglycerol. *Can. J. Biochem. Cell Biol.* **63**: 919–926.
- Igal, R. A., and R. A. Coleman. 1996. Acylglycerol recycling from triacylglycerol to phospholipid, not lipase activity, is defective in neutral lipid storage disease fibroblasts. *J. Biol. Chem.* **271**: 16644–16651.
- Esko, J. D., and C. R. Raetz. 1980. Autoradiographic detection of animal cell membrane mutants altered in phosphatidylcholine synthesis. *Proc. Natl. Acad. Sci. USA.* **77**: 5192–5196.
- Esko, J. D., M. M. Wermuth, and C. R. Raetz. 1981. Thermolabile CDP-choline synthetase in an animal cell mutant defective in lecithin formation. *J. Biol. Chem.* **256**: 7388–7393.
- Cui, Z., M. Houweling, M. H. Chen, M. Record, H. Chap, D. E. Vance, and F. Terce. 1996. A genetic defect in phosphatidylcholine biosynthesis triggers apoptosis in Chinese hamster ovary cells. *J. Biol. Chem.* **271**: 14668–14671.
- Sleight, R., and C. Kent. 1983. Regulation of phosphatidylcholine biosynthesis in mammalian cells. I. Effects of phospholipase C treatment on phosphatidylcholine metabolism in Chinese hamster ovary cells and LM mouse fibroblasts. *J. Biol. Chem.* **258**: 824–830.
- Sweitzer, T. D., and C. Kent. 1994. Expression of wild-type and mutant rat liver CTP:phosphocholine cytidyltransferase in a cytidyltransferase-deficient Chinese hamster ovary cell line. *Arch. Biochem. Biophys.* **311**: 107–116.
- Wang, Y., T. D. Sweitzer, P. A. Weinhold, and C. Kent. 1993. Nuclear localization of soluble CTP:phosphocholine cytidyltransferase. *J. Biol. Chem.* **268**: 5899–5904.
- Lykidis, A., I. Baburina, and S. Jackowski. 1999. Distribution of CTP:phosphocholine cytidyltransferase (CCT) isoforms. Identification of a new CCTbeta splice variant. *J. Biol. Chem.* **274**: 26992–27001.
- Zhang, D., W. Tang, P. M. Yao, C. Yang, B. Xie, S. Jackowski, and I. Tabas. 2000. Macrophages deficient in CTP:phosphocholine cytidyltransferase-alpha are viable under normal culture conditions but are highly susceptible to free cholesterol-induced death. Molecular genetic evidence that the induction of phosphatidyl-

- choline biosynthesis in free cholesterol-loaded macrophages is an adaptive response. *J. Biol. Chem.* **275**: 35368–35376.
22. Houweling, M., Z. Cui, and D. E. Vance. 1995. Expression of phosphatidylethanolamine N-methyltransferase-2 cannot compensate for an impaired CDP-choline pathway in mutant Chinese hamster ovary cells. *J. Biol. Chem.* **270**: 16277–16282.
  23. Esko, J. D., and K. Y. Matsuoka. 1983. Biosynthesis of phosphatidylcholine from serum phospholipids in Chinese hamster ovary cells deprived of choline. *J. Biol. Chem.* **258**: 3051–3057.
  24. Esko, J. D., M. Nishijima, and C. R. Raetz. 1982. Animal cells dependent on exogenous phosphatidylcholine for membrane biogenesis. *Proc. Natl. Acad. Sci. USA.* **79**: 1698–1702.
  25. Lloyd-Davies, K. A., and D. N. Brindley. 1975. Palmitate activation and esterification in microsomal fractions of rat liver. *Biochem. J.* **152**: 39–49.
  26. Coleman, R. A. 1992. Diacylglycerol acyltransferase and monoacylglycerol acyltransferase from liver and intestine. *Methods Enzymol.* **209**: 98–104.
  27. Polokoff, M. A., and R. M. Bell. 1977. Characterization of liver cholic acid coenzyme A ligase activity. Evidence that separate microsomal enzymes are responsible for cholic acid and fatty acid activation. *J. Biol. Chem.* **252**: 1167–1171.
  28. Coleman, R. A., and R. M. Bell. 1980. Selective changes in enzymes of the sn-glycerol 3-phosphate and dihydroxyacetone-phosphate pathways of triacylglycerol biosynthesis during differentiation of 3T3-L1 preadipocytes. *J. Biol. Chem.* **255**: 7681–7687.
  29. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Med. Sci.* **37**: 911–917.
  30. Igal, R. A., M. C. Pallanza de Stringa, and I. N. Tacconi de Gómez Dumm. 1994. Simple method for quantitation of neutral and polar lipids by TLC and fluorescence emission. *Int. J. Bio-Chromatogr.* **1**: 137–142.
  31. Sambrook, J., and D. W. Russell. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 7.1–7.94.
  32. Lowry, O. R., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275.
  33. Labarca, C., and K. Paigen. 1980. A simple, rapid, and sensitive DNA assay procedure. *Anal. Biochem.* **102**: 344–352.
  34. Merrill, A. H., Jr., S. Gidwitz, and R. M. Bell. 1982. Facile enzymatic synthesis of fatty acylcoenzyme A thioesters. *J. Lipid Res.* **23**: 1368–1373.
  35. Chang, Y. Y., and E. P. Kennedy. 1967. Biosynthesis of phosphatidyl glycerophosphate in *Escherichia coli*. *J. Lipid Res.* **8**: 447–455.
  36. Igal, R. A., S. Wang, M. R. Gonzalez-Baro, and R. A. Coleman. 2001. Mitochondrial glycerol phosphate acyltransferase directs the incorporation of exogenous fatty acids into triacylglycerol. *J. Biol. Chem.* **276**: 42205–42212.
  37. Pelech, S. L., P. H. Pritchard, D. N. Brindley, and D. E. Vance. 1983. Fatty acids promote translocation of CTP:phosphocholine cytidyltransferase to the endoplasmic reticulum and stimulate rat hepatic phosphatidylcholine synthesis. *J. Biol. Chem.* **258**: 6782–6788.
  38. Meegalla, R. L., J. T. Billheimer, and D. Cheng. 2002. Concerted elevation of acyl-coenzyme A:diacylglycerol acyltransferase (DGAT) activity through independent stimulation of mRNA expression of DGAT1 and DGAT2 by carbohydrate and insulin. *Biochem. Biophys. Res. Commun.* **298**: 317–323.
  39. Schaffer, J. E. 2003. Lipotoxicity: when tissues overeat. *Curr. Opin. Lipidol.* **14**: 281–287.
  40. Unger, R. H., and L. Orci. 2002. Lipoapoptosis: its mechanism and its diseases. *Biochim. Biophys. Acta.* **1585**: 202–212.
  41. Waite, K. A., and D. E. Vance. 2000. Why expression of phosphatidylethanolamine N-methyltransferase does not rescue Chinese hamster ovary cells that have an impaired CDP-choline pathway. *J. Biol. Chem.* **275**: 21197–21202.
  42. Jackowski, S., J. Wang, and I. Baburina. 2000. Activity of the phosphatidylcholine biosynthetic pathway modulates the distribution of fatty acids into glycerolipids in proliferating cells. *Biochim. Biophys. Acta.* **1483**: 301–315.
  43. Karim, M., P. Jackson, and S. Jackowski. 2003. Gene structure, expression and identification of a new CTP:phosphocholine cytidyltransferase beta isoform. *Biochim. Biophys. Acta.* **1633**: 1–12.
  44. Clement, J. M., and C. Kent. 1999. CTP:phosphocholine cytidyltransferase: insights into regulatory mechanisms and novel functions. *Biochem. Biophys. Res. Commun.* **257**: 643–650.
  45. Coleman, R. A., T. M. Lewin, C. G. Van Horn, and M. R. Gonzalez-Baro. 2002. Do long-chain acyl-CoA synthetases regulate fatty acid entry into synthetic versus degradative pathways? *J. Nutr.* **132**: 2123–2126.
  46. Coleman, R. A., T. M. Lewin, and D. M. Muoio. 2000. Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. *Annu. Rev. Nutr.* **20**: 77–103.
  47. Stals, H. K., W. Top, and P. E. Declercq. 1994. Regulation of triacylglycerol synthesis in permeabilized rat hepatocytes. Role of fatty acid concentration and diacylglycerol acyltransferase. *FEBS Lett.* **343**: 99–102.
  48. Murphy, D. J., and J. Vance. 1999. Mechanisms of lipid-body formation. *Trends Biochem. Sci.* **24**: 109–115.
  49. Brasaemle, D. L., B. Rubin, I. A. Harten, J. Gruia-Gray, A. R. Kimmel, and C. Londos. 2000. Perilipin A increases triacylglycerol storage by decreasing the rate of triacylglycerol hydrolysis. *J. Biol. Chem.* **275**: 38486–38493.
  50. Brasaemle, D. L., T. Barber, N. E. Wolins, G. Serrero, E. J. Blanchette-Mackie, and C. Londos. 1997. Adipose differentiation-related protein is an ubiquitously expressed lipid storage droplet-associated protein. *J. Lipid Res.* **38**: 2249–2263.
  51. Verkade, H. J., D. G. Fast, A. E. Rusinol, D. G. Scraba, and D. E. Vance. 1993. Impaired biosynthesis of phosphatidylcholine causes a decrease in the number of very low density lipoprotein particles in the Golgi but not in the endoplasmic reticulum of rat liver. *J. Biol. Chem.* **268**: 24990–24996.