Lipoprotein lipase synthesis and secretion: effects of concentration and type of fatty acids in adipocyte cell culture[1]

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Abstract Fatty acids (FA) were added to differentiating chick adipocytes to study their effects on the synthesis and secretion of avian lipoprotein lipase (LPL). Oleate (18:1n-9), eicosapentaenoate (EPA, 20:5n-3), and linoleate (18:2n-6) were complexed to fatty acid-free bovine serum albumin (BSA) and separately added to cells in RMPI-1640 media containing 0.5% delipidated hen serum. LPL secretion in the presence of 10 U/ml heparin was used as a means of estimating LPL synthesis. FA from 50 μM to 165 μM depressed LPL secretion for cells exposed to 20:5n-3 and 18:2n-6. The decrease in adipocyte LPL secretion was only observed with chronic administration of FA. In other experiments, the rate of LPL synthesis was estimated by incorporation of radiolabel (Tran ^{35}S-label^{TM}) in cells exposed to 50 μM FA. Total radioactivity incorporated into LPL, expressed as a percentage of total trichloroacetic acid (TCA)-precipitable radioactivity, was not affected in cells fed 18:1n-9 but was significantly decreased for cells fed 20:5n-3 (45%) or 18:2n-6 (67%) relative to controls given equimolar BSA (33 μM). Abundance of LPL message in similarly treated cells also decreased for cells incubated with 20:5n-3 or 18:2n-6 (35%) relative to controls and 18:1n-9-treated cells. A decrease in adipocyte LPL secretion was also observed with administration of lipoprotein (d < 1.006 g/ml) enriched in n-3 and n-6 fatty acids. LPL secretion of cells incubated with n-3 enriched lipoprotein at 50 μM and 75 μM triglyceride fatty acid equivalents was significantly greater than that of cells incubated with n-6-enriched lipoprotein. Interestingly, at the same concentrations of triglyceride fatty acids, lipoproteins enriched with n-9 fatty acids had no effect on LPL secretion relative to controls. ▪ These studies document that in cultured avian adipocytes, LPL secretion, synthesis, and level of message are decreased by chronic administration of n-3 and n-6 fatty acids. In contrast, in adipocytes supplemented with oleic acid there was no effect on LPL synthesis and secretion.—**Montalto, M. B., and A. Bensadoun.** Lipoprotein lipase synthesis and secretion: effects of concentration and type of fatty acids in adipocyte cell culture. *J. Lipid Res.* 1993. **34:** 397–407.

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Lipoprotein lipase (LPL) is primarily synthesized and secreted by subendothelial cells of adipose, heart, and skeletal muscle (1, 2) and is responsible for the hydrolysis of the triglyceride-rich core of circulating chylomicrons and very low density lipoproteins (1–7). Control of LPL gene expression by hormonal factors has been shown to be tissue-specific (5, 7). Insulin increases gene expression in adipocytes (8–11). In heart cells cAMP increases cellular LPL dramatically (12); in adipocytes the effect of cAMP is more complex. Short term exposure to cAMP decreases LPL synthesis and secretion (12, 13) whereas long term treatment (24 h) increases the secretion of the lipase.

Epidemiological and clinical research has suggested that a diet rich in polyunsaturated fatty acids may decrease the incidence of cardiovascular disease by lowering plasma triglyceride and very low density lipoprotein concentrations (14, 15). Long chain fatty acids derived from fish oil, mainly eicosapentaenoic acid (EPA, 20:5n-3), have been shown to be an important factor in the hypotriglyceridemic response to diets containing fish oil (14, 16). It is possible that fatty acids in fish oils and other oils affect the synthesis and secretion of LPL; changes in LPL activity would influence the clearance of triglycerides.

The primary objective of this study was to test the hypothesis that enriching cellular lipids in specific fatty acids has an effect on the synthesis and secretion of LPL in cultured adipocytes. Adipocytes were enriched in oleic acid (18:1n-9), eicosapentaenoic acid (EPA, 20:5n-3), or linoleic acid (18:2n-6) by either incubation of fatty acids complexed to fatty acid-free BSA or by incubation with

lipoproteins enriched in a specific fatty acid. This paper shows that adipocyte LPL synthesis and secretion is lowered by fatty acids of the n–6 and n–3 families, whereas fatty acids of the n–9 family have little or no effect. The lowering of LPL synthesis and secretion is more accentuated with n–6 than n–3 fatty acids.

MATERIALS AND METHODS

Materials

Oleate (18:1n–9), linoleate (18:2n–6), and other fatty acids used for supplementation of media and fatty acid methyl ester standards were from Nu-Chek Prep, Inc. Eicosapentaenoic acid, EPA (20:5n–3) was purchased from Cayman Chemical Co. MaxEPA® oil was donated by R. P. Scherer Co., Troy, MI. Tran[35]S-label[TM] was purchased from ICN Radiobiochemicals. Radiolabeled nucleotide for mRNA solution hybridization assay ([α-^{32}P]dCTP; 3,000 Ci/mmol or 111 TBq/mmol) was obtained from Dupont NEN®.

Adipocyte preparation and media conditions

Preadipocytes from 3-week White Leghorn chicks were isolated aseptically and plated in sterile culture dishes as previously described (17). Cells were plated in RPMI 1640 (25 mM HEPES) media with 15% fetal bovine serum (day 0) and the media were replaced on day 1. On day 3 or 4, medium was replaced by RPMI 1640 (25 mM HEPES) containing either 0.5% or 1% delipidated hen serum plus antibiotic supplements (50 μg/ml penicillin, 50 μg/ml streptomycin, 100 μg/ml neomycin PSN, GIBCO), 2 mM L-glutamine, 10 μg/ml insulin, 1 mg/ml D-glucose, 0.5% methyl cellulose, 20 μM dexamethasone (delipidated hen serum medium, DHSM), or DHSM supplemented with specific fatty acids that were complexed to essentially fatty acid-free bovine serum albumin (BSA, Sigma A 7030) in RPMI 1640.

For each experiment, unless indicated, BSA was maintained at 33 μM such that only the ratio of fatty acids to BSA varied. Fatty acid to BSA ratios of 1:1 to 3:1 are within physiological levels (18). Eighty percent of the medium was replaced every 2 to 3 days with DHSM or DHSM supplemented with the desired fatty acid concentration. In cell experiments in which lipoproteins were used as the source of fatty acid, DHSM plus lipoproteins were used. With the exception of one set of experiments, fatty acids and lipoprotein-containing media were removed from the adipocytes 48 h prior to harvest, and replaced with 0.5% or 1.0% DHSM unless indicated. This medium change was carried out so that differences observed could be ascribed to differences in endogenous cellular lipids rather than to acute effects of unesterified fatty acids added in the medium. In several experiments,

as indicated, the glucose concentration was reduced from 295 mg/dl (normal media conditions) to 25 mg/dl for 48 h prior to harvest. Initially, the intent of the glucose reduction was to decrease the flux of fatty acyl-CoA intermediates to triglyceride and thereby increase the intracellular pool of free fatty acid. However, with avian adipocytes, the glucose concentration (25 vs. 295 mg/dl) had no statistically significant effect on LPL secretion in the presence of heparin in control or fatty acid-treated adipocytes.

Hen serum was delipidated by organic solvent extraction (19). Delipidated hen serum was analyzed for fatty acid content by gas chromatography and was found to be fatty acid-free.

Fatty acid complexed to BSA in RPMI 1640

Fatty acids were converted to their sodium salt and complexed to fatty acid-free BSA in RPMI 1640 medium (20). After incubation, the fatty acid-BSA complex in RPMI 1640 medium was adjusted to pH 7.2, filter-sterilized with a 0.22-μm filter, and stored at 4°C. Expected concentrations of fatty acids bound to BSA were verified by gas chromatography and the concentrations were adjusted accordingly.

Gavage administration of oils and lipoprotein isolation from roosters

Adult White Leghorn roosters (~ 3 kg) were fasted approximately 24 h and then given 5 ml of olive oil, MaxEPA, or safflower oil by gavage. Just prior to delivery of the oils, LPL activity was inhibited by intravenous wing vein injection of goat antiserum raised against chicken adipose LPL (6, 21). Blood was collected by heart puncture and the d < 1.006 g/ml fraction was isolated, washed through BSA containing sucrose step gradients, and concentrated by flotation as described previously (6, 21). Triglyceride concentration of the lipoproteins was determined enzymatically (ReagentSet® Triglycerides GPO, Boehringer Mannheim 701912); cholesterol (21) and total fatty acid (22) composition were determined after lipid extraction (23) by gas chromatography.

Adipocyte harvest and collected fractions

Cells were harvested within 12–14 days of plating in culture medium. On the day of harvest, cells were incubated for 5 h with or without 10 U/ml heparin (Sigma H 3125). After incubation, cells were placed on ice and the media were saved for measurement of LPL by ELISA. The cell surface was washed with 2 ml ice-cold 0.15 M NaCl, 5 mM phosphate, pH 7.4 (PBS) to remove residual media. To measure cell surface-associated LPL, 1 ml of ice-cold RPMI 1640 containing 10 U/ml heparin was added to each 60-mm dish and incubated for 10 min at 4°C on ice with shaking (Lab-Line Instrument, Inc.). The heparin

wash was collected and the cell surface was washed again with ice-cold PBS. LPL in the combined fractions is defined as cell surface associated LPL. Cells were harvested in ice-cold lysis buffer A (4 mM CHAPS, 3.3 U/ml heparin, and 50 mM NH_4OH, pH 8.1) or, when isotope was used, lysis buffer B (0.75 M NaCl, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, 10 U/ml heparin, and 10 mM HEPES, pH 7.4). LPL in this fraction is defined as the cellular LPL. Both lysis buffers contained antiproteolytic agents (1 μg/ml leupeptin, 1 μg/ml chymostatin, and 1 μg/ml pepstatin, 2 μg/ml antipain, 10 μg/ml benzamidine, and 10 U/ml Trasylol®). Cells collected in the respective buffers were then sonicated at 60 watts for 15 sec in a Braun-sonic 1510 probe sonicator equipped with a 4-mm microprobe. Thus, three fractions were collected at each harvest: cell extract, wash buffer containing 10 U/ml heparin (containing surface-associated LPL), and incubation medium (plus or minus 10 U/ml heparin). For a given cell batch the coefficient of variation for LPL secretion was 7.5 ± 4% for 14 determinations. Cell triglycerides increased with days in culture. For each experiment, days during which cells were fed fatty acid-containing media are indicated in the legends. Aliquots of cell extract harvested in lysis buffer A were used for determination of DNA (24), triglyceride content (Reagentset® Triglycerides GPO, Boehringer Mannheim 70192), and protein (25). LPL in various fractions was measured by a sensitive ELISA. Details of this assay have been described previously (26). Minor modifications follow. Microtiter plates were coated overnight with 0.5 μg/well of affinity-purified immunoglobulins in 200 μl of 0.02% NaN_3, 0.1 M carbonate–bicarbonate buffer, pH 9.6. Best conditions for sample incubations were overnight incubations at 4°C in 1 M NaCl, 1% BSA, 0.05% Tween-20, 10 mM phosphate, pH 7.4. The standard curve ranged from 0.03 to 1.4 ng. For seven assays, the slope of the standard curve was 0.708 ± 0.05 OD unit/1 ng LPL and the correlation coefficient was 0.998 ± 0.002.

LPL secretion and synthesis

In some instances secretion of LPL in the presence of heparin (10 U/ml) was determined; medium was changed to medium containing 10 U/ml heparin. Enzyme mass in the medium was then determined by ELISA 5 h after medium change. In previous work with avian adipocytes we have shown that heparin addition to the medium displaces LPL from the cell surface and inhibits internalization and degradation of the enzyme (17). Heparin did not affect the synthesis rate. In the presence of heparin, LPL secretion approximated the synthetic rate (17, 26). This method was used as a rapid and sensitive means of estimating synthetic rates under a variety of cell culture conditions. LPL synthesis was also measured directly by pulsing cells for 10 min with 100 μCi/dish of Tran ^{35}S-label™ in complete RPMI 1640 containing only 5 μM

methionine. Labeled LPL was isolated by immunoadsorption as described in detail by Cupp, Bensadoun, and Melford (17).

Lipid extraction and determination of fatty acid content of cultured chick adipocytes

Cells were collected after incubation for 5 h as described above and the lipids were extracted by a modified version of published methods (23, 27). Lipids extracted from cells were saponified, the nonsaponifiable lipids were removed, and after acidification the fatty acids were extracted in hexane. For intracellular nonesterified fatty acid analyses, the dried lipids were resuspended in chloroform and spotted on activated silica gel 60 thin-layer chromatography (TLC) plates (E. M. Merck) that had been pre-run in chloroform–methanol 2:1 (v/v). The bands containing the free fatty acids were scraped from the plate and eluted with three consecutive 5-ml washes of chloroform–methanol 9:1 (v/v). Free fatty acids eluted from the silica gel were converted to methyl ester derivatives as described below.

Fatty acid analysis

Methyl ester derivatives of dried fatty acid extracts were prepared with ethereal diazomethane (N-methyl-N-nitroso-p-toluenesulfonamide, Diazald®, Aldrich) or 14% boron trifluoride–methanol (Sigma B 1252) as described (22) and quantified with a Hewlett-Packard 5890A gas chromatograph equipped with a capillary column, a flame ionization detector, and a 3396A integrator. Capillary columns used were 30 m long and had an 0.32 mm inside diameter and a 0.25 micron film thickness; stationary phases used were either 50% cyanopropyl–50% methyl (J&W Scientific, DB-23) or Carbowax® polyethylene glycol 20 M, Stabilwax (Restek, 10624). Hydrogen was used as carrier gas and nitrogen was used for the make-up gas. Inlet and detector zones were set at 260°C. The oven temperature was initially set at 160°C followed by an increase of 2°C/min to 202°C for 21 min followed by an increase of 8°C/min to 235°C for 5 min.

LPL mRNA measurements

RNA was isolated by the acid guanidinium thiocyanate–phenol–chloroform extraction technique (28). Levels of message for LPL mRNA were determined by a DNA-excess solution hybridization assay (29) with a 212 nucleotide single-stranded DNA probe encoding residues 157 to 228 of avian LPL (30). A genomic clone in M13mp18 (clone 389) including exon V sequences (31) served as a template for primer extension using [α-^{32}P]dCTP for probe labeling. Isolation of the single-stranded labeled probe was essentially as described previously by Williams et al. (29). Each sample was analyzed in quadruplicate hybridizations with 60 μg of total adipocyte RNA per reaction. The hybridization reactions were

conducted in a 1.5-ml microcentrifuge tube at 68°C for 60 h in 0.03 M Tris-HCl, pH 7.0, 0.3 M NaCl, 0.02 M EDTA, 5 μg/ml yeast RNA, and 100 μg/ml denatured salmon sperm DNA. Increasing concentrations of template were used to generate the standard curve for the hybridization assay. The concentration of LPL mRNA in the samples was determined by interpolation from a standard curve ranging from 0 to 2,000 pg per tube.

Statistical methods

One-way analysis of variance (ANOVA) was conducted to determine whether there were significant differences ($P < 0.05$) among treatments. If significant differences were determined by ANOVA, a two-sample t-test was conducted to determine the significance ($P < 0.05$) of differences between treatments. For the studies with lipoproteins enriched in specific fatty acids, LPL secretion was regressed against fatty acid composition for the aggregate set of data (one control and nine treatments). Families of fatty acids and selected ratios of fatty acid families were specified as independent variables in the regression equation. An R^2 statistic was generated and was used as a criterion for selecting the subset of independent variables that best explained the change in LPL synthesis.
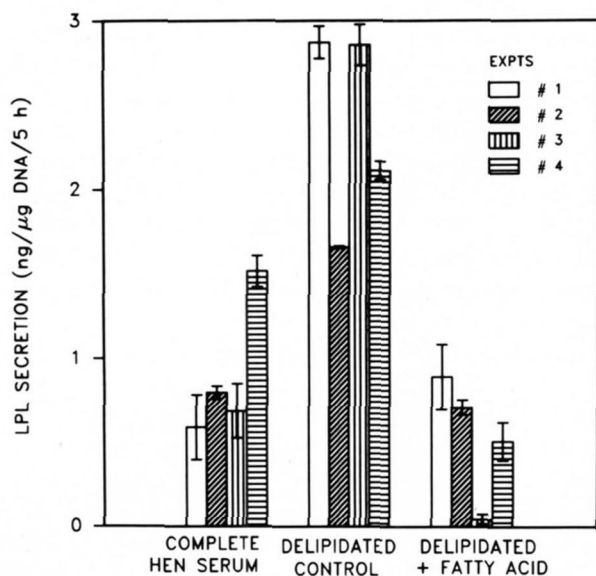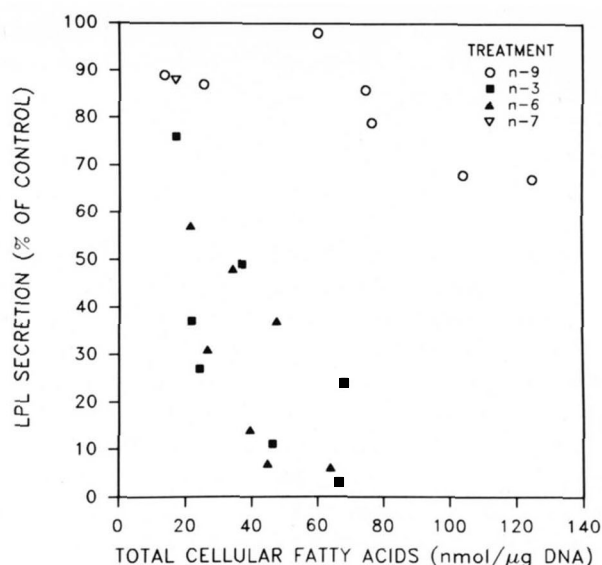

Fig. 2. Effect of total cellular fatty acids and type of fatty acid on lipoprotein lipase secretion. Adipocytes were incubated with fatty acid (FA)-containing media for 7 days at 100 μM FA and 33 μM BSA in 1.0% DHSM. FA-containing medium was removed 48 h prior to harvest and replaced with 0.5% DHSM. Cells were incubated for 5 h in the presence of 10 U/ml heparin. Lipoprotein lipase (LPL) present in the media was measured by ELISA and corrected for cellular DNA level per dish. Total cellular fatty acids (esterified and nonesterified) from similarly treated dishes were measured by gas chromatography. Each point represents the mean from three pools of three 60-mm dishes.

RESULTS

Effects of lipids in serum on the secretion of LPL

In order to evaluate the effects of lipids in hen serum on LPL secretion rate, adipocytes were incubated in complete hen serum medium, delipidated hen serum medium (DHSM), and DHSM with fatty acids complexed to BSA. The presence of lipids in hen serum markedly reduced the secretion of LPL from avian adipocytes compared to cells incubated with DHSM (**Fig. 1**). In four separate experiments with different cell batches, adipocytes that were incubated in DHSM secreted significantly more LPL compared to cells incubated in complete hen serum media ($P < 0.002$). The addition of 18:2n–6 or 20:5n–3 to cells incubated in DHSM resulted in a decrease in LPL synthesis similar to that of cells incubated in complete hen serum media.

Effect of the type of fatty acid and total cell fatty acid pool on adipocyte LPL secretion

To evaluate whether the total cellular fatty acid (esterified and nonesterified) was associated with the decline in LPL secretion, cells were lipid-extracted and the total fatty acid pool was determined by gas chromatography. In four separate experiments, as total cellular fatty acids in treated cells increased, LPL secretion decreased, suggesting an inverse relationship of LPL secretion and total cellular fatty acids (**Fig. 2**). The decreased secretion of LPL

Fig. 1. Effect of lipids in hen serum on the secretion of lipoprotein lipase. Adipocytes were incubated in medium containing either 0.5% complete hen serum (not delipidated), delipidated hen serum, or delipidated hen serum supplemented with linoleic acid at 140 μM (Expt #1), 200 μM (Expt #2), or eicosapentaenoic acid 187 μM (Expt #3), 200 μM (Expt #4). BSA was constant at 33 μM for all experiments. Cells were exposed to treatments for 9 days. Lipoprotein lipase (LPL) present in the media was determined by ELISA after 5 h incubation in the presence of lipid at the indicated level and 10 U/ml heparin. Bars represent mean ± SD lipoprotein lipase (LPL) for three replicates of two 60-mm dishes.

was most influenced by the family of fatty acid that was used during the incubation period. It is striking that with cells supplemented with n–6 or n–3 fatty acids LPL secretion declined markedly as cellular fatty acids per cell increased. This relationship was greatly attenuated in cells supplemented with n–9 fatty acids. The fatty acid composition of the adipocyte was evaluated to verify that the exogenous fatty acids were incorporated into cellular lipids. Levels of palmitic acid (16:0) remained relatively constant across all cell treatments (14–20 mol%). The predominant fatty acid in control cells incubated with 0.5% DHSM was 18:1n–9 at 40 mol%. The fatty acid used for supplementation was determined to be the predominant cellular fatty acid. Levels of 18:2n–6 were low in controls (< 1 mol%) and all cell treatments except for those cells supplemented with 18:2n–6 (27 mol%). Eicosapentaenoic acid could only be detected in cells exposed to 20:5n–3 (37 mol%).

Intracellular nonesterified fatty acid levels were determined for a similar set of cells described in Fig. 2 in which cells were exposed to 0.5% DHSM and 50 μM fatty acid. Data were expressed as nmol intracellular free fatty acid per 100 μg DNA from four replicate samples of one 100-mm dish per treatment. Intracellular free fatty acid levels for controls (286 ± 28) and treatments, 18:1n–9 (271 ± 32), 20:5n–3 (283 ± 18), and 18:2n–6 (264 ± 41), were not significantly different. However, molar composition of the nonesterified fatty acid pool was enriched in the specific fatty acid added to the cell medium. For control cells and for cells exposed to 50 μM fatty acids, the mole % 18:1n–9, 18:2n–6 and 20:5n–3 were 10, 3, 0; 23, 0.6, 0; 8, 10, 0; and 5, 0.7, 17, respectively, for control cells and for cells fed 18:1n–9, 18:2n–6, and 20:5n–3.

Effect of fatty acid concentration on adipocyte LPL secretion and cellular triglyceride

The effect of fatty acid concentration was studied in the range of 33–165 μM. In cells exposed to 18:1n–9 there was no effect of fatty acid concentration on LPL secretion in the presence of heparin (**Fig. 3**). At each concentration
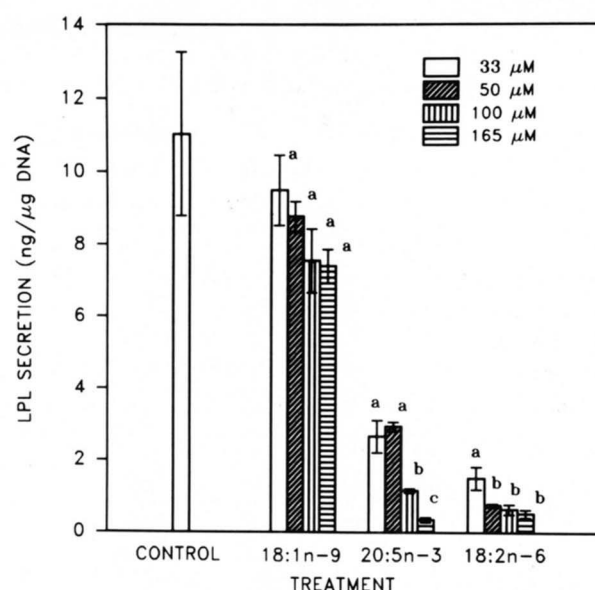

Fig. 3. Effect of concentration and type of fatty acid on lipoprotein lipase secretion. Adipocytes were exposed to indicated concentrations of fatty acid treatment in 0.5% delipidated hen serum medium (DHSM) for 9 days. Media for all treatments contained 33 μM BSA. Fatty acid-containing media were removed 48 h before harvest and replaced with 0.5% DHSM containing 25 mg/dl glucose. Cells were incubated for 5 h in the presence of 10 U/ml heparin and the lipoprotein lipase (LPL) present in the media was measured by ELISA and corrected for cellular DNA level per dish. Each bar represents the mean ± SD from three pools of two 60-mm dishes. Bars with different letters within treatments indicate significant differences, $P < 0.05$.

tested, 18:1n–9-treated cells secreted more LPL than cells exposed to 20:5n–3 or 18:2n–6 ($P < 0.01$). A significant decrease in LPL secretion was observed with 20:5n–3 ($P < 0.02$) or 18:2n–6 ($P < 0.02$) at 33 μM relative to controls and 18:1n–9 cells. At 50 μM fatty acid, cells incubated with 18:1n–9 accumulated significantly more triglyceride than cells exposed to 20:5n–3, $P = 0.009$ or 18:2n–6, $P = 0.012$ (**Table 1**). Despite the fact that there were no significant differences in cellular triglyceride among treatments at 165 μM fatty acid, LPL secretion was significantly modified by the type of fatty acid (Fig.

TABLE 1. Effect of fatty acid concentration on adipocyte triglyceride

	Fatty Acid Concentration				
	0 μM	33 μM	50 μM	100 μM	165 μM
		cellular triglyceride (μg/μg DNA)			
Control	14.0 ± 1.7[a]				
18:1n–9		16.5 ± 1.9[a]	18.3 ± 2.4[a]	26.1 ± 1.7[a]	31.7 ± 1.7[a]
20:5n–3		6.5 ± 1.8[b]	7.6 ± 1.8[b]	22.1 ± 0.4[a]	35.6 ± 1.4[a]
18:2n–6		9.6 ± 2.2[a,b]	9.2 ± 1.7[b]	15.6 ± 2.1[b]	32.8 ± 2.6[a]

Cellular triglyceride levels for adipocytes described in Fig. 3. were measured. Cells were exposed to indicated levels of fatty acid complexed to 33 μM BSA for 9 days. Forty eight hours prior to harvest, the fatty acids were removed and cells were incubated in 0.5% delipidated hen serum media containing 25 mg/dl glucose. Means ± SD of three replicate samples of two 60-mm dishes. Values with different superscripts within columns are significantly different, $P < 0.05$. Control levels were compared to treatment at 33 μM fatty acid.

3) used in the culture medium. From these observations we conclude that the composition of intracellular lipids may have a greater effect on adipocyte LPL secretion than the quantity of triglyceride per cell.

Effect of fatty acid on LPL mass in cell extracts, cell surface, and incubation media containing heparin

Previous experiments concentrated mainly on the effects of various fatty acids on LPL secretion. **Fig. 4** summarizes results of an experiment that measured, in addition, intracellular and cell surface-associated LPL. Compared to cells exposed to DHSM or media supplemented with 50 μM 18:1n–9 or 20:5n–3, cells exposed

Fig. 5. Effect of fatty acid treatment on the incorporation of Trans ^{35}S-label® into lipoprotein lipase. Adipocytes were incubated in 0.5% DHSM supplemented with 50 μM FA and 33 μM BSA for 8 days. FA-containing media were removed 48 h prior to harvest and replaced with 0.5% DHSM containing 25 mg/dl glucose. A 10 min pulse of Tran[35]S-label® (100 μCi/dish) was delivered to cells. Labeled lipoprotein lipase (LPL) was isolated and quantified from cell extracts by immunoadsorption followed by electrophoresis and fluorography. Bars represent means ± SD of three pools of eight 60-mm dishes. Bars with different letters are significantly different, $P < 0.05$.
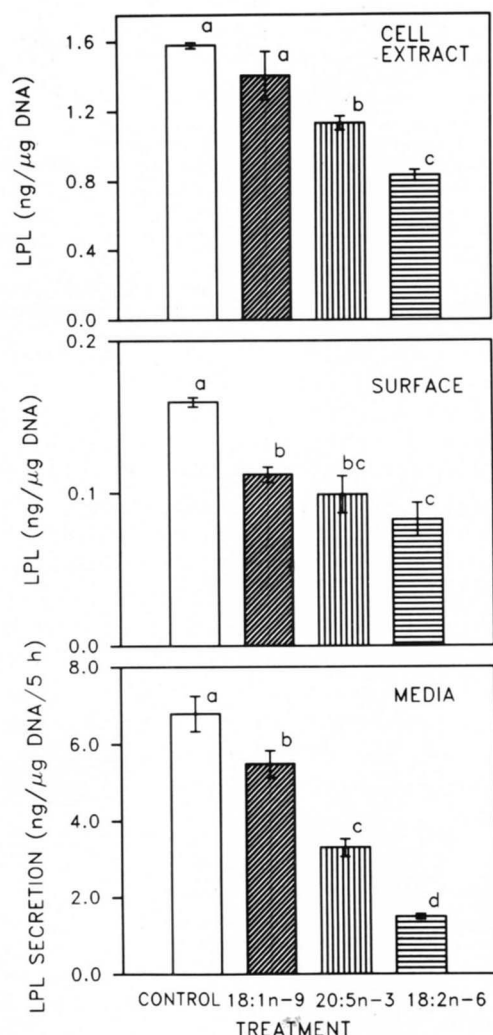

Fig. 4. Effects of type of fatty acid on lipoprotein lipase in cell extract, cell surface and media. Adipocytes were incubated in 0.5% DHSM supplemented with 50 μM FA and 33 μM BSA for 8 days. FA-containing media were removed 48 h prior to harvest and replaced with 0.5% DHSM containing 25 mg/dl glucose. Cells were incubated for 5 h in the presence of 10 U/ml heparin. Lipoprotein lipase (LPL) present in the three pools was measured by ELISA and corrected for cellular DNA level per dish. Each bar represents the mean ± SD from four pools of four 60-mm dishes. Bars within LPL fractions with different letters indicate significant differences, $P < 0.05$.
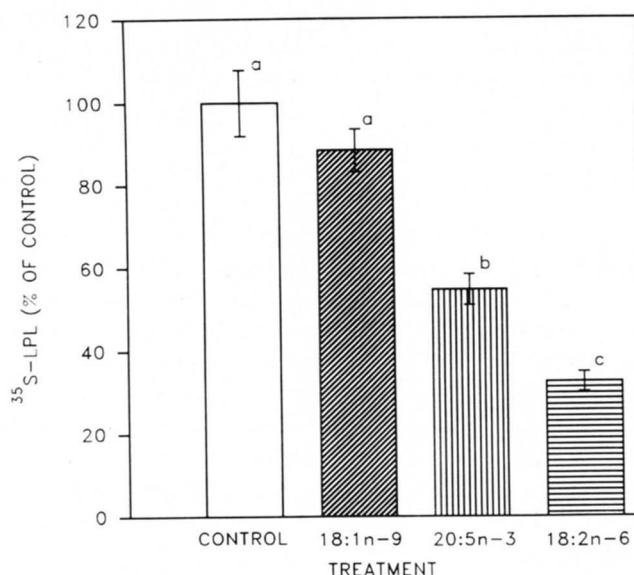
to 50 μM 18:2n–6 had the least amount of LPL present in cell extracts, cell surface and media. In addition, cells fed 20:5n–3 had more cellular lipase ($P < 0.05$) and secreted more enzyme ($P < 0.05$) than those fed 18:2n–6.

LPL synthesis

Using the same batch of cells as those used in experiments described in the previous paragraph, rates of LPL synthesis were evaluated directly by incorporation of Tran ^{35}S-label™ into LPL during a 10-min pulse. Radiolabeled LPL was isolated from cell extracts by immunoprecipitation followed by electrophoresis and fluorography (17, 26). Adipocytes exposed to 18:1n–9 did not have a different rate of LPL synthesis ($P = 0.13$) compared to controls (**Fig. 5**). Compared to control and 18:1n–9 cells, the rate of LPL synthesis was decreased for cells exposed to 20:5n–3 ($P = 0.012$ and $P = 0.003$, respectively) or 18:2n–6 ($P = 0.005$ and $P = 0.004$, respectively). LPL synthetic rate was greater for cells exposed to 20:5n–3 compared to 18:2n–6 ($P = 0.003$).

Adipocyte LPL mRNA abundance: effect of fatty acid supplementation

One possibility for the observed decrease in LPL synthesis among treatments is a decrease in the abundance of message for the enzyme. To explore this possibility, levels of LPL mRNA were determined by a DNA-excess

solution hybridization assay (29) using a single-stranded 212 nucleotide DNA probe (30) for chicken adipose LPL. Results of the hybridization assay are presented as a percent of control levels (194 ± 46 fmol/µg RNA per µg DNA/dish) (**Fig. 6**). Control and 18:1n-9-treated cells had significantly greater levels of message compared to cells incubated with 20:5n-3 ($P = 0.012$ and $P = 0.002$, respectively) and 18:2n-6 ($P = 0.012$ and $P = 0.002$, respectively). Levels of message were not different between cells exposed to 20:5n-3 or 18:2n-6.

Effect of acute fatty exposure on LPL secretion in differentiated adipocytes

Adipocytes were incubated in 0.5% DHSM up to the day of harvest. Cells were then preincubated for 3 h in 0.5% DHSM containing either 33 µM BSA or 33 µM BSA supplemented with 264 µM 20:5n-3 or 264 µM 18:2n-6; this was followed by replacement of the media with the same composition with or without heparin (10 U/ml) for 5 h. In spite of the high fatty acid concentration there were no significant differences in the secretion of LPL in the presence or absence of heparin (data not shown). It appears that the effect of 18:2n-6 and 20:5n-3 fatty acids on LPL secretion requires extended exposure
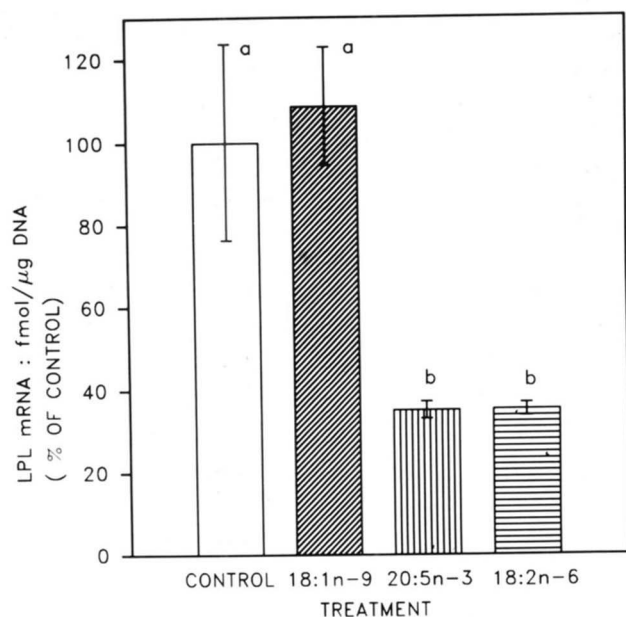

Fig. 6. Effect of fatty acid treatment on lipoprotein lipase mRNA. Adipocytes were incubated in 0.5% DHSM supplemented with 50 µM FA and 33 µM BSA for 9 days. FA-containing media were removed 48 h prior to harvest and replaced with 0.5% DHSM containing 25 mg/dl glucose. Levels of message for lipoprotein lipase (LPL) were measured by DNA-excess solution hybridization assay and expressed per cell DNA content (open bars). Bars for level of message represent mean percent ± SD from four pools of six 100-mm dishes. Significant differences ($P < 0.05$) for mRNA measurements are indicated by different letters above hatched bars, and open bars, respectively. Representative data from three experiments.

of adipocytes to these fatty acids for periods greater than 8 h.

Experiments with lipoproteins enriched with specific fatty acids

Lipoproteins (d < 1.006 g/ml) enriched in specific fatty acids were used as another vehicle for delivery of fatty acids to cells. The lipoproteins isolated for study reflected the composition of the oil used for gavage feeding of roosters used to produce triglyceride-rich lipoproteins (**Table 2**). The mole percent ratio of lipoprotein cholesterol to triglyceride fatty acid (mean ± SD) was 2.2 ± 0.1 for olive oil treatment, 3.4 ± 0.2 for MaxEPA treatment, and 2.9 ± 0.01 for safflower oil treatment. Lipoprotein triglyceride concentrations were determined enzymatically and expressed in micromolar triglyceride fatty acid levels for comparison to previous experiments with isolated fatty acids. Administration of lipoprotein with a triglyceride fatty acid concentration ranging from 10 µM to 75 µM resulted in a significant increase in cellular triglyceride (**Table 3**) and a significant decrease in the secretion of LPL in the presence of heparin relative to control adipocytes incubated with 1% DHSM (**Fig. 7**). At all levels of triglyceride fatty acids, LPL secretion was greatest in adipocytes incubated with lipoproteins derived from olive oil ($P < 0.02$). At 10 µM triglyceride fatty acid, there were no significant differences in LPL secretion between MaxEPA and safflower oil treatments. At triglyceride fatty acid levels of 50 µM and 75 µM, there were significant differences ($P < 0.005$) among treatments (olive > MaxEPA > safflower) in the secretion of LPL in the presence of heparin. The increase in cellular triglycerides for cells fed MAXEPA or safflower lipoproteins was associated with a decrease in LPL secretion (**Fig. 8**). Quantitation of the composition of the cellular lipid suggests that LPL synthesis decreases as the molar ratio of fatty acids of the n-3 and n-6 family $\Sigma(n\text{-}3_i + n\text{-}6_j)$ increases relative to fatty acids of the n-7 and n-9 family $\Sigma(n\text{-}7_k + n\text{-}9_l)$ (**Fig. 9**). The ratio of all n-3 and n-6 fatty acids relative to all n-7 and n-9 fatty acids was the fatty acid grouping most explanatory with respect to LPL secretion with an R^2 statistic of 0.86.

DISCUSSION

These studies document that LPL synthesis and secretion in differentiated avian adipocytes is influenced by chronic administration of fatty acids or serum lipids in the incubation medium. Fatty acids from the n-9 family had little or no effect on adipocyte LPL synthesis and secretion, but these two rates were markedly decreased for cells incubated with n-6 and n-3 fatty acids. Linoleic acid is more effective than 20:5n-3 in decreasing LPL synthesis and secretion when the concentration of fatty acid in the

TABLE 2. Composition of oils and lipoproteins derived from gavage administration of oils to roosters

Fatty Acids	Oil			Lipoprotein		
	Olive	MaxEPA	Safflower	Olive	MaxEPA	Safflower
	mol %					
n–3	0.3	30.2	0.2	1.6	17.1	1.8
n–6	9.9	3.7	72.0	15.3	13.3	50.5
n–9	73.1	11.8	15.1	60.3	21.7	22.1
Saturates	15.0	39.6	11.8	20.5	38.1	23.8

Fatty acids were analyzed by gas chromatography, summed and expressed as mol percent. Fatty acids (all-*cis* configuration) identified for the various families were as follows; n-3 (18:3, 18:4, 20:3, 20:5, 22:3, 22:5, and 22:6); n-6 (18:2, 18:3, 20:2, 20:3, 20:4, 22:2, and 22:4); n-9 (18:1, 18:2, 20:1, 22:1, and 24:1). Saturated fatty acids were composed of 14, 16, 18, 20, 22, and 24 carbon units.

medium is 50 μM or less (Figs. 3, 4, and 5). In some experiments adipocytes were treated with triglyceride-rich lipoproteins obtained from donor animals fed olive oil, MaxEPA, or safflower oil; the objective was to supply adipocytes an alternative source of fatty acids, and also to conduct an empirical comparison of oils commonly used in human diets. The experiments clearly show that feeding the fatty acid mix present in olive oil lipoproteins at different levels had no effect on LPL secretion. However, exposing cells to 50 and 75 μM triglyceride fatty acids from MaxEPA or safflower oil lipoproteins inhibited LPL secretion and the effect was more accentuated with safflower lipoproteins than with MaxEPA lipoproteins. With respect to effect of specific fatty acids, the experiments should be interpreted cautiously as the respective mole % values of n-9, n-3, and n-6 fatty acids in the three lipoproteins preparations were not equal (Table 2). We could, however, analyze cellular fatty acids and ask whether there was a relationship between cellular fatty acid composition and LPL secretion. For cells supplemented with the MaxEPA or the safflower oil lipoproteins, LPL secretion was highly correlated (Fig. 9) with the fraction $(n-3_i + n-6_i)/(n-7_i + n-9_i)$. This fraction accounted for 86% of the variation in LPL secretion: although the sample size is limited, this is an indication that the mole % values, of n-3 and n-6 fatty acids in the cellular lipids are likely a significant determinant of LPL secretion.

Total cellular fatty acids are probably not as significant a modulator of LPL secretion as the mole % of n-6 and n-3 fatty acids. The effect of total cellular fatty acids on LPL secretion was not significant when cells were fed n-9 fatty acids. In experiments where cells were fed n-3 or n-6 fatty acids, total cell fatty acids increased as higher molar concentrations of these fatty acids were added in media; concurrently, LPL secretion decreased (Fig. 8). The apparent effect of cell lipids may simply be due to the fact that as cell lipids are increasing, the mole % of n-3 and n-6 is also increasing. A detailed statistical analysis factoring out the effects of cellular fatty acid composition and total cell lipids will need to be conducted on a larger sample size. It is not known whether LPL synthesis in cultured adipocytes can be increased by depletion of the cellular triglyceride pool. Experiments in humans consuming a mixed fatty acid diet suggest that adipose LPL biosynthesis, in vivo, can be increased by depletion of the cellular triglyceride pool. Kern (32) has reported increases in adipose tissue LPL activity, enzyme mass and message after very obese individuals consuming a mixed fatty acid diet maintained significant weight loss.

TABLE 3. Effect of lipoprotein triglyceride fatty acid concentration and composition on cellular triglyceride

	Lipoprotein Triglyceride Fatty Acids			
	0 μM	10 μM	50 μM	75 μM
	cellular triglyceride (μg/μg DNA)			
Control	1.6 ± 0.4[a]			
Olive		3.1 ± 0.2[b]	5.1 ± 0.1[a]	6.3 ± 0.3[a]
MaxEPA		2.7 ± 0.3[b,c]	4.8 ± 0.3[a]	6.2 ± 0.04[a]
Safflower		2.3 ± 0.3[a,c]	3.2 ± 0.1[b]	5.0 ± 0.03[b]

Adipocytes were incubated in 1% delipidated hen serum medium for 9 days and treatments received the indicated micromolar levels of triglyceride fatty acids. Means ± SD from three or four replicates of two 60-mm dishes. Values with different superscripts within columns are significantly different, $P < 0.05$. Control levels were compared to treatment at 10 μM lipoprotein triglyceride fatty acid.
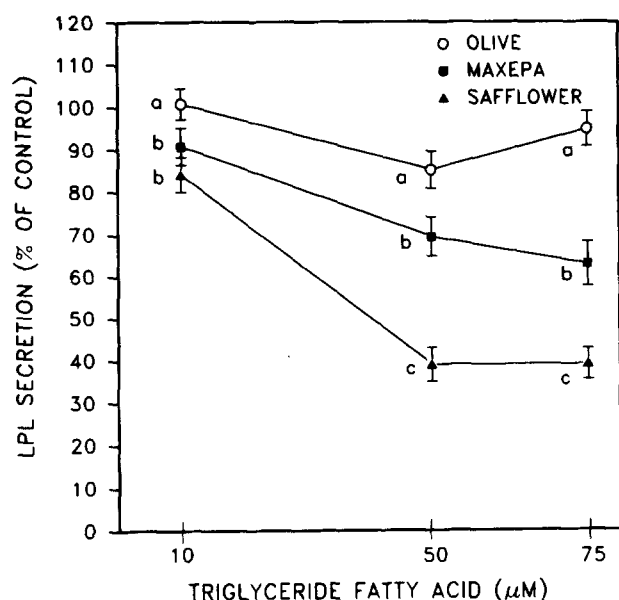
Fig. 7. Effect of lipoproteins derived from olive, MaxEPA, and safflower oil-fed roosters on lipoprotein lipase secretion in cultured adipocytes. Adipocytes were incubated with lipoprotein (d < 1.006 g/ml) at the indicated triglyceride fatty acid concentrations for 9 days in 1% DHSM. Lipoprotein-containing media were removed 48 h prior to harvest. LPL present in 5-h incubation media containing 10 U/ml heparin was determined by ELISA and corrected for cellular DNA level per dish. Data points represent mean percent ± SD from four replicates of two 60-mm dishes. Representative data from three experiments.
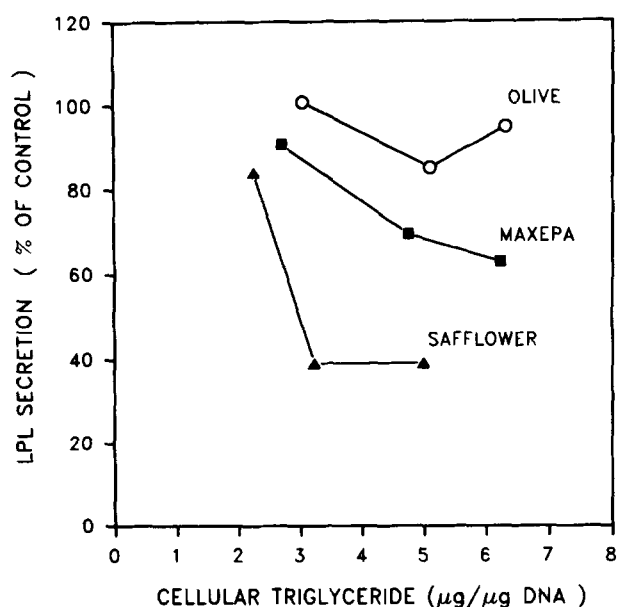

Fig. 8. Cellular triglyceride and lipoprotein lipase secretion. Data from the same set of cells described in Table 5 and Fig. 7 plotted to show the relationship of cellular triglyceride accumulation and LPL secretion. Cellular triglyceride and LPL secretion data were adjusted for cellular DNA content. Secretion was measured for 5 h in the presence of heparin (10 U/ml).
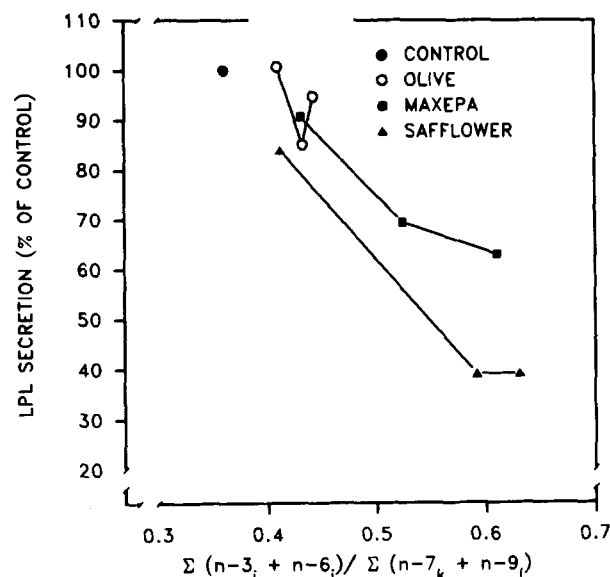

Fig. 9. The percent molar ratio of all n–3 and n–6 fatty acids relative to all n–7 and n–9 fatty acids plotted against lipoprotein lipase (LPL) secretion data from experiment in Fig. 7. Data points for fatty acid ratios represent analysis by gas chromatography of two replicates of three 60-mm dishes.

Regulation of adipocyte LPL secretion by n–3 and n–6 fatty acids is a long-term effect. Acute exposure of adipocytes to an 8:1 fatty acid to bovine serum albumin ratio had no effect on LPL secretion in cultured adipocytes. If, as indicated earlier, LPL synthesis rate responds to changes in composition of cellular fatty acids, a minimal period of exposure to a given fatty acid is required to modify the composition of cellular fatty acids sufficiently to cause a detectable change in synthesis or secretion. Previous studies have shown that the reduced postprandial triglyceridemic effect of n–3 fatty acids is due to chronic intake of fish oil rather than acute administration (33). Acute exposure of adipocytes to fatty acids did decrease the level of LPL present on the cell surface. This finding was consistent with a report in which fatty acids were shown to release LPL bound to the surface of endothelial cells (34).

We have shown that cells incubated with 18:1n–9 accumulate large amounts of triglyceride relative to cells incubated with n–6 and n–3 fatty acids. Oleic acid has been shown to be a potent stimulator of triglyceride synthesis and secretion of triglyceride-rich lipoproteins by CaCo-2 cells (35); in cultured parenchymal cells and microsomes from rat liver, oleic acid was also a potent stimulator of triglyceride synthesis (36). Eicosapentaenoic acid has been shown to reduce hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A-1,2-diacylglycerol acyltransferase (36) and has also been shown to inhibit VLDL cholesteryl ester secretion by reducing the activity of acyl-CoA:cholesterol acyltransferase (37).

Intracellular free fatty acids were first proposed to regulate LPL synthesis by Nikkilä (38), however, the methods of detection for LPL were indirect and enzyme mass was not detected. Our data show that LPL synthesis and secretion were not directly linked to the total intracellular concentration of nonesterified fatty acids. The present data suggest that fatty acid composition of the cellular lipids may be a more significant regulator. There is a growing body of information suggesting that fatty acids may be regulating the expression of several genes directing the synthesis of proteins related to lipid metabolism. Fatty acids or their derivatives have been shown to activate expression of the gene coding for an adipocyte-specific fatty acid-binding protein, aP2 in Ob 1771 cells (39). The induction of the aP2 gene by fatty acid was shown to be unique to adipose cells (40). After the present work was completed, a report by Ntambi (41) appeared showing that feeding mice tripalmitin, tristearin, or triolein had no effect on liver stearyl-CoA desaturase 1 mRNA, whereas feeding trilinolein, trilinolenin, and triarachidonin repressed induction of the mRNA.

Fatty acid-binding proteins are known to reversibly bind nonesterified fatty acids (42, 43) and to modulate the activities of enzymes involved in lipid metabolism (44, 45). We propose that the binding of fatty acids to specific fatty acid-binding protein(s) results in a competent *trans*-acting factor that may regulate gene expression by interacting with a "fatty acid response element" on the LPL gene. Differences in binding constants with different fatty acids (46–50) could explain the observed fatty acid specificity. In the cytosol of chicken adipose tissue, a 14.4 kDa fatty acid-binding protein showed greater binding activity to linoleate than to oleate (46). The degree to which the avian adipose fatty acid binding protein bound n–3 fatty acids was not determined. The present work justifies the search for a "fatty acid response element" and for fatty acid-specific *trans*-factors that may be expressed in a tissue-specific manner. ▪

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