Oleic Acid Inhibits Cholesteryl Esterase and Cholesterol Utilization for Testosterone Synthesis in Mouse Leydig Cells

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We have observed that nonesterified fatty acids (NEFA) inhibit testosterone synthesis in response to luteinizing hormone (LH) in mouse Leydig cells, possibly by affecting cholesterol utilization or endogenous concentrations. We have now studied the influence of oleic acid (OA) on the cellular content of cholesterol, hydrolysis of cholesterol esters, and steroidogenesis in isolated mouse Leydig cells. OA (700 μmol/L added with fatty acid–free [FAF] albumin, 3 g/dL) significantly ($P < .025$) reduced testosterone production in response to LH (10 ng/mL), total cholesterol concentrations of Leydig cells and the culture medium, and cholesteryl esterase activity in the cytosol and mitochondria. We also studied the effects of OA on steroidogenesis and cellular cholesterol concentrations after treatments to increase cellular cholesterol. OA at lower concentrations (5 μmol/L with albumin, 0.1 g/dL) or low-density lipoprotein ([LDL] 4 μg protein/mL) increased cellular cholesterol ($P < .01$) without affecting basal steroidogenesis. These treatments failed to reverse the inhibitory ($P < .05$) effect of OA on testosterone synthesis following LH stimulation, but did significantly ($P < .01$) increase cellular cholesterol. In summary, OA appears to inhibit testosterone synthesis by inhibiting cholesteryl esterase activity.

LEYDIG CELLS in the testes of mammals synthesize testosterone from cholesterol derived from endogenous synthesis and plasma lipoproteins. The contribution of cholesterol used for testosterone synthesis from these sources varies among species, but human and mouse Leydig cells use similar sources.[1,2] A cellular cholesterol pool generated by these sources is transported to the inner mitochondrial membrane, where the cholesterol side-chain cleavage enzyme converts it to pregnenolone.[1-5]

Studies on the influence of plasma nonesterified fatty acids (NEFA) on the steroidogenesis of testosterone have been reported. Meikle et al[6] demonstrated that triglycerides and either a mixture of NEFA or individual fatty acids, particularly oleic acid (OA), inhibited testosterone production in isolated murine Leydig cells in response to luteinizing hormone (LH). The site of inhibition of testosterone synthesis appeared to affect cholesterol utilization because inhibition could be reversed by 22-hydroxycholesterol, a substrate used for pregnenolone formation. This cholesterol substrate does not require the effects of cyclic adenosine monophosphate.[6,7] These inhibitory effects of NEFA on testosterone synthesis in Leydig cells were reversible, were not characteristic of detergent influences, and could not be attributed to influences on LH/human chorionic gonadotropin binding to receptors.[6] Goodfriend et al[8] subsequently reported that fatty acids modulated the synthesis of aldosterone in the adrenal cortex of the rat. Since studies reported by Meikle et al[6] and Goodfriend et al[8] indicated that OA was a potent inhibitor of steroidogenesis, it was selected for the current investigation.

In addition, several clinical disorders and situations, including diabetes mellitus, obesity, fasting, and ingestion of meals high in fat, elevate serum NEFA and are also associated with suppression of plasma testosterone.[6,9-20] It remains to be determined whether the observed reduction in serum testosterone is caused by the elevation of NEFA. The previous studies suggested that NEFA at concentrations within the physiologic range (<1,500 μmol/L)[21,22] influenced steroidogenesis in human and mouse Leydig cells. To investigate this possibility, we conducted studies in mouse isolated Leydig cells to determine if OA, which is abundant in the diet and serum,[21,22] alters cholesterol concentrations and cholesteryl esterase activity that could affect cholesterol utilization for steroidogenesis. Our results suggest that OA blocks cholesterol utilization independently of effects on Leydig cell cholesterol concentrations.

MATERIALS AND METHODS

Materials for In Vitro Studies in Leydig Cells

Minimal essential medium (MEM) containing a 1.36-mmol/L Ca^{2+} concentration was purchased from Irvine Scientific (Santa Ana, CA), and highly purified human LH (9,991 IU/mg; lot #AFP 4360B) was obtained from the National Hormone and Pituitary Program. Fatty acid–free (FAF) bovine serum albumin, human low-density lipoprotein (LDL), and cholesterol standards were purchased from Sigma Chemical (St Louis, MO). Percoll was obtained from Pharmacia (Piscataway, NJ). All unlabeled steroids were obtained from Steraloids (Wilton, NH). Tritiated steroids of high specific activity (50 to 140 Ci/mmol) were purchased from Dupont–New England Nuclear (Boston, MA).

Isolation and Incubation of Mouse Leydig Cells

Mature Swiss-Webster adult male mice from Simonsen (Gilroy, CA) were fed chow as described previously by Meikle et al[6]; the mice were killed by cervical dislocation. Testes were removed using sterile technique, decapsulated, and shaken at the rate of 60 times per minute at room temperature in MEM (10 mL per testes) containing 0.1% bovine serum albumin (FAF), penicillin 100 U/mL, streptomycin 50 μg/mL, and 25 mmol/L HEPES, pH 7.4, for 10 minutes. Then the mixture was filtered through one layer of sterile gauze and centrifuged at 80 *g* for 10 minutes at 20°C, resuspended in MEM (1 to 2 testes per mL), layered over a continuous gradient of Percoll and Earle salts (0% to 90%), and centrifuged for 20 minutes at 800 × *g* at 20°C. The purified Leydig cells were found in a band corresponding to a Percoll concentra-

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tion of 38% to 52% (vol/vol), and it resulted in greater than 75% purity determined by staining for 3β-hydroxysteroid dehydrogenase[23] and electron microscopy, which showed fragments of seminiferous tubular cells besides Leydig cells. Before use, the cells were washed twice; trypan blue exclusion tests indicated greater than 90% viability.

The cells were incubated using Falcon Multiwell culture plates (Becton Dickinson, Lincoln Park, NJ). The final incubation volume was 500 μL MEM with either 3 g/dL (used to solubilize 700 μmol/L OA) or 0.1 g/dL (used with 5 to 20 μmol/L OA) bovine serum albumin (FAF), which included the various substrates. LH was added at a concentration of 10 ng/mL unless otherwise stated. All treatments were added at the beginning of each timed experiment. Culture plates were incubated with 95% air/5% CO_2 at 37°C for 4 or 12 hours. No pH changes occurred during incubations. Typical cell numbers ranged from 1 to 4 10^5 cells per well. Incubations were concluded by addition of 0.5 mL ice-cold radioimmunoassay buffers for 10 minutes.

Triplicate 10-μL aliquots of incubation media from each well were assayed for testosterone by radioimmunoassay as described elsewhere.[6] Testosterone production in each well ranged from 5 to 500 ng/10^6 cells, depending on the stimulator added. All testosterone assays from each aliquot were measured in triplicate, and the coefficient of variation was typically between 5% and 10%. Representative results from these experiments are reported. Blank control values were less than 5 pg per tube, and interassay and intraassay coefficients of variation for the assay were 10% or less. Cross-reactions with other steroids were insignificant. Each reported experiment was repeated on at least three occasions.

Measurement of Cholesterol Concentrations

Leydig cells from each well were scraped free and centrifuged. The resulting pellet from each well representing 1 to 4 × 10^5 cells was suspended in 0.2 mL 100-mmol/L Tris buffer, pH 7.24, and sonicated for 10 seconds. Protein determination was made on a 50-μL aliquot using the Bio-Rad protein assay (Bio-Rad, Richmond, CA), and the remaining sample (150 μL) was extracted three times with 3 mL hexane. These extracts were collected and then evaporated to dryness and redissolved in 0.4 mL hexane. Total cholesterol level was measured by hydrolyzing samples with alcoholic KOH (15.2% wt/vol, 0.5 mL 1-mmol/L KOH in 95% ethanol) at 60°C for 2 hours. Hydrolyzed cholesteryl oleate (>95%) was used as a quantitative standard, and 5α-cholestane was used as an internal recovery standard.[24-26] Samples containing cholesterol were measured by gas chromatography (Hewlett-Packard model 5830A 6C, Boise, ID) using a modification of the method described by Murray and Nelson.[21] Neutral cholesteryl esterase activity was measured in cytosol and mitochondria by methods reported previously.[27,28]

Statistical Analysis

Data are reported as the mean ± SE unless stated otherwise. ANOVA with repeated measures was performed to analyze the effect of treatment on cellular cholesterol or testosterone levels, and Student's *t* test was used to determine statistical significance ($P < .05$).

RESULTS

Effects of OA on Testosterone Synthesis and Leydig Cell Cholesterol Concentrations

Treatment of Leydig cells with OA (700 μmol/L with FAF albumin, 3 g/dL) for either 4 or 12 hours reduced testosterone ($P < .05$) production (Figs 1A and 2A) in response to LH. In contrast, incubation of Leydig cells with OA for 12 hours but not for 4 hours significantly ($P < .05$) reduced cholesterol concentrations (Figs 1B and 2B). OA but not LH treatment for 12 hours decreased ($P < .05$) the concentration of cholesterol in the culture medium (Fig 2C), suggesting that OA influences the movement of cholesterol from Leydig cells into the incubation medium. The reduction in Leydig cell cholesterol concentrations in response to LH indicated its use for steroidogenesis (Fig 2B). These results show that OA appeared to inhibit

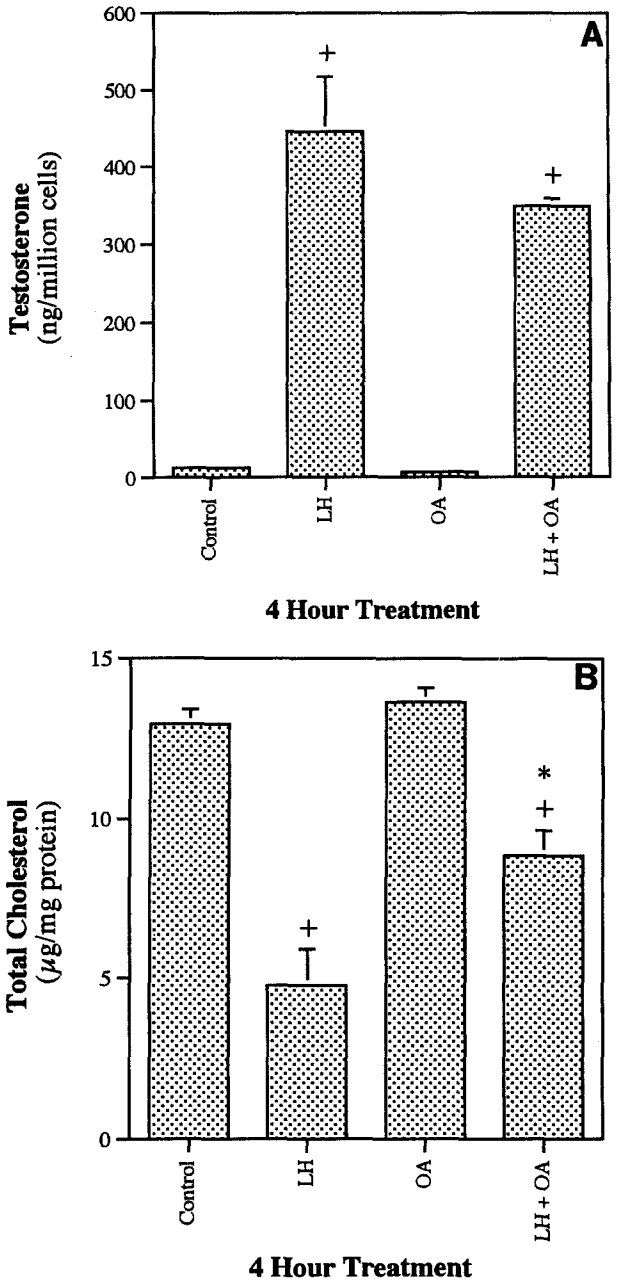

Fig 1. Effect of a 4-hour incubation with OA (700 μmol/L with FAF albumin 3 g/dL) on Leydig cell testosterone synthesis (A) and total cholesterol concentration (B). Values are the mean ± SE; n = 6. $^+P < .05$ *v* controls; $^*P < .05$, OA *v* LH 10 ng/mL + OA.

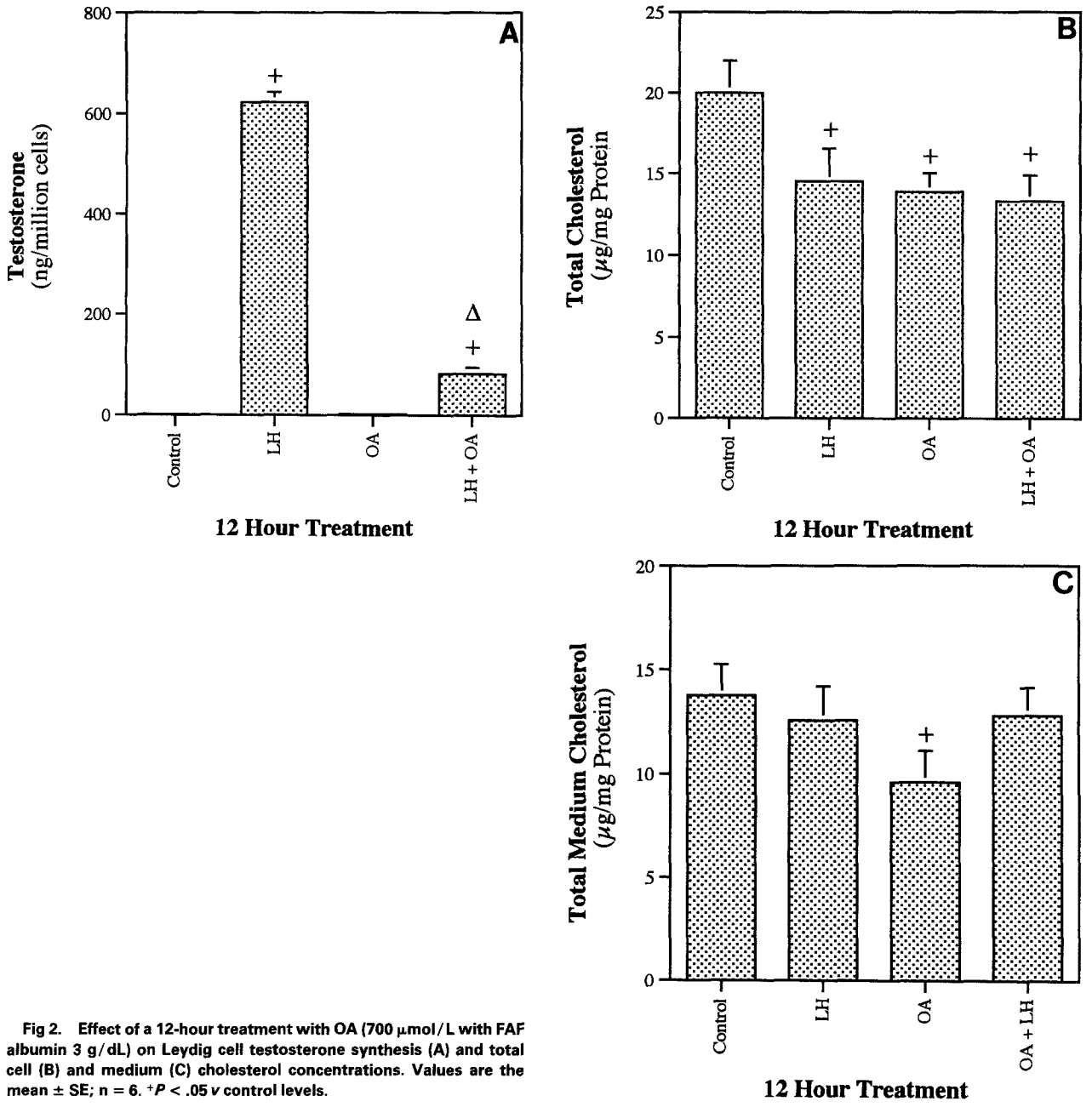

Fig 2. Effect of a 12-hour treatment with OA (700 μmol/L with FAF albumin 3 g/dL) on Leydig cell testosterone synthesis (A) and total cell (B) and medium (C) cholesterol concentrations. Values are the mean ± SE; n = 6. $^{+}P < .05$ *v* control levels.

cholesteryl hydrolase and cholesterol synthesis when incubated for 12 hours. Incubations of Leydig cells with OA for 12 but not for 4 hours blocked the reduction in cholesterol in Leydig cells in response to LH.

We then determined if elevating Leydig cellular cholesterol concentrations would reverse the effects of OA on testosterone synthesis. We elevated cellular cholesterol by two treatments: one with low-dose OA and another with LDL. OA concentrations at 5 μmol/L (bovine serum albumin FAF 0.1 g/dL) significantly ($P < .05$) decreased Leydig cell testosterone synthesis (Fig 3A) in response to LH despite significantly ($P < .05$) increasing the cholesterol concentrations (Fig 3B). Comparable results were observed with 10 or 20 μmol/L OA (data not shown). Without added LH, basal testosterone synthesis was unaffected. These findings indicate that low concentrations of OA inhibit testosterone synthesis in Leydig cells while elevating cholesterol concentrations. This suggests that low concentrations of OA may inhibit cholesterol mobilization for steroidogenesis stimulated by LH.

Effect of LDL Cholesterol on Leydig Cell Cholesterol Concentration and on Reversing the Inhibitory Effects of OA

In experiments with LDL, dose-response effects of the lipoprotein on testosterone synthesis and cholesterol concentrations of the cells were studied. LDL at a concentration of 4 μg protein/mL was selected as the second method to elevate cellular cholesterol because it did not affect basal

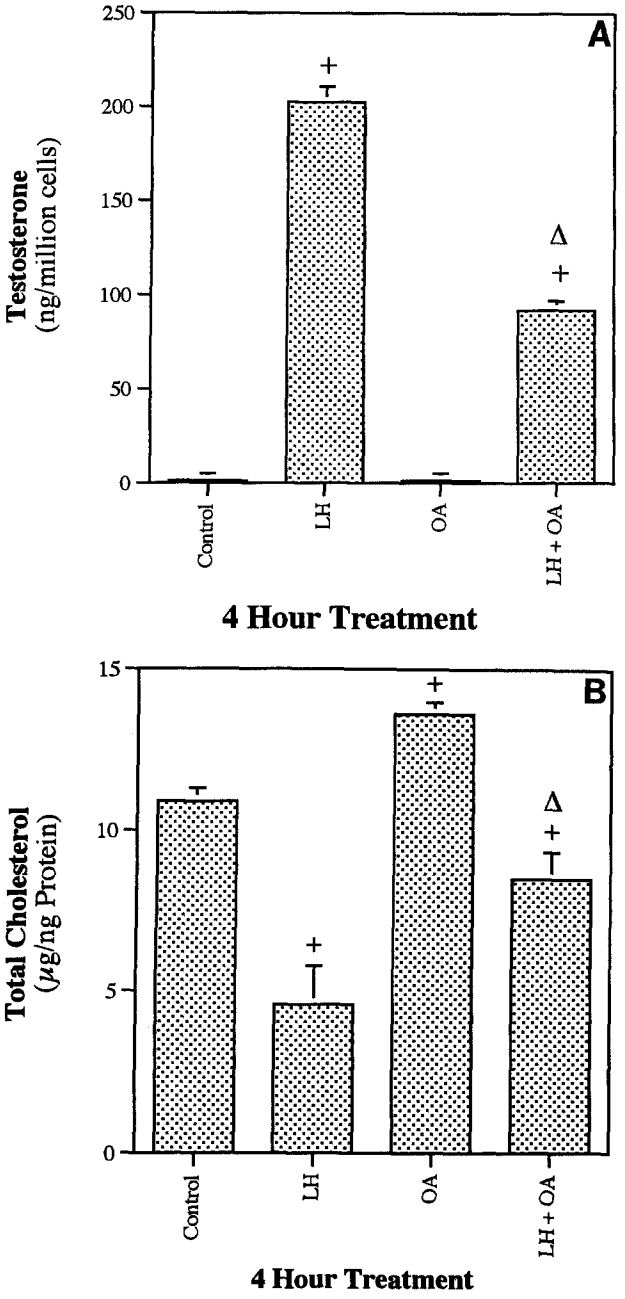

Fig 3. Effect of a 4-hour incubation with OA (5 μmol/L with FAF albumin 0.1 g/dL) on Leydig cell testosterone synthesis (A) and cholesterol concentration (B). Values are the mean ± SE; n = 6. (A) $^{+}P < .05$, testosterone synthesis with LH alone *v* LH plus OA. (B) $^{+}P < .05$ *v* control levels; $^{\Delta}P < .05$, OA without LH *v* OA with LH.

or LH-stimulated production of testosterone without OA. LDL treatment of Leydig cells did not alter ($P < .05$) basal and post–LH-stimulated testosterone synthesis (Fig 4A), but increased cholesterol concentrations (Fig 4B). However, LH treatment of Leydig cells loaded with cholesterol by treatment with LDL decreased ($P < .05$) cholesterol concentrations (Fig 4B).

We then examined whether elevating Leydig cell cholesterol concentrations by LDL treatment affected testosterone production in response to LH and OA. Incubation with OA (5 μmol/L) reduced ($P < .05$) Leydig cell synthesis of testosterone in response to LH and LDL (Fig 4A). OA increased ($P < .05$) cholesterol concentrations in Leydig

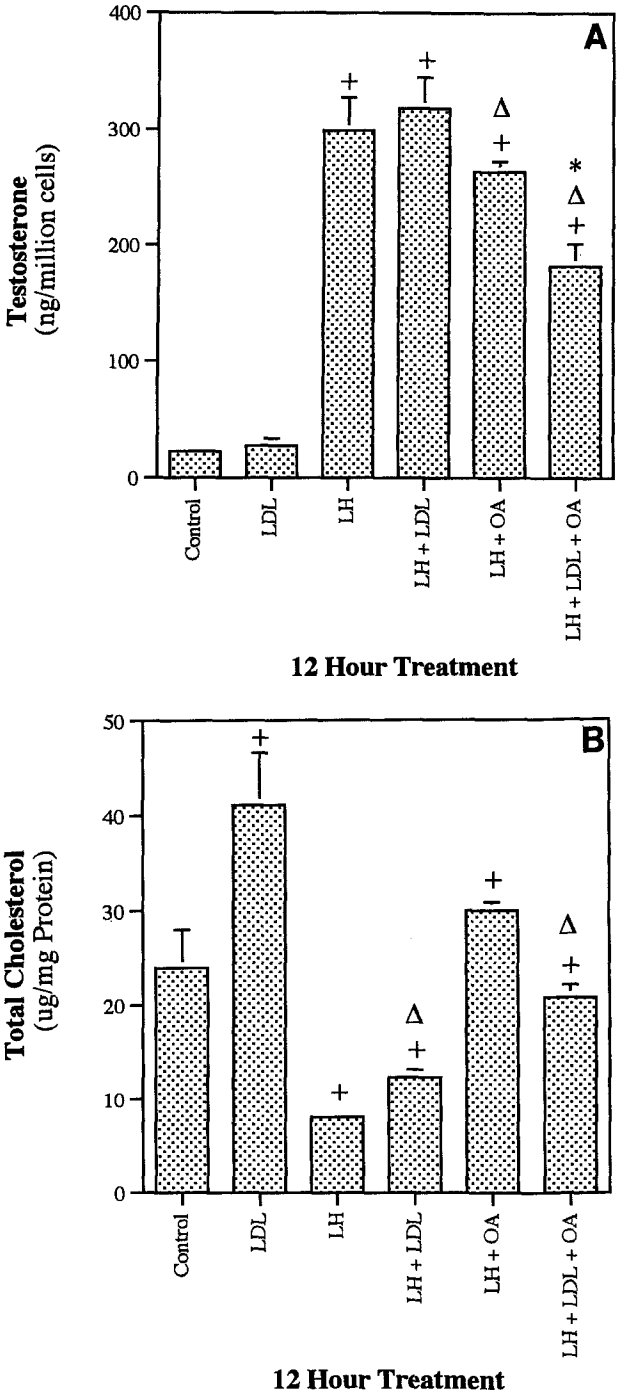

Fig 4. Effect of a 12-hour treatment with LDL (4 μg/mL protein) and OA on Leydig cell testosterone synthesis (A) and cholesterol concentration (B). Values are the mean ± SE; n = 6. (A) $^{+}P < .05$, treatments *v* controls; $^{\Delta}P < .05$, LH *v* other treatments; $^{*}P < .05$, LH + OA *v* LH + LDL + OA. (B) $^{+}P < .05$ *v* control levels; $^{\Delta}P < .05$, LH *v* LH + OA and LH + LDL + OA.

cells above the levels seen with LH and LDL (Fig 4B). Although LDL treatment failed to reverse the inhibitory effect of OA on testosterone synthesis (Fig 4A), LH significantly reduced Leydig cell cholesterol concentrations in cells also treated with LDL and OA versus LH and OA (Fig 4B). These results suggest that OA (5 μmol/L) reduces LH-stimulated cholesterol mobilization and thereby decreases testosterone synthesis.

Effect of OA on Neutral Cholesteryl Ester Hydrolysis

To study the effect of OA on cholesterol esterase, hydrolysis of cholesterol oleate in both cytosol (Fig 5A) and mitochondria (Fig 5B) was studied. Hydrolysis increased significantly ($P < .05$) in response to LH in isolated Leydig cells treated for 12 hours before isolation of cytosol and mitochondria. OA (700 μmol/L with FAF albumin 3 g/dL) significantly ($P < .025$) decreased hydrolysis in response to LH, but not less than the basal rate of the control. These results may suggest that OA inhibits cholesteryl esterase activity, which would affect cholesterol utilization.

DISCUSSION

We have used three strategies to assess the influence of NEFA on utilization of cholesterol for testosterone synthesis in isolated mouse Leydig cells. The first strategy was to measure the concentration of cholesterol in Leydig cells treated with NEFA with and without LH. Depletion of cholesterol from steroid-producing cells is known to reduce their capacity to produce steroid hormones in response to trophic hormones.[29-32] The second was to elevate the levels of cholesterol, the substrate for steroidogenesis, in Leydig cells in an attempt to overcome the inhibitory effect of OA on testosterone production in response to LH. Repleting cholesterol in cholesterol-depleted Leydig cells has been shown to reverse the inhibition of steroidogenesis caused by depletion of cholesterol.[5,30-33] The third was to measure the effects of OA on hydrolysis of cholesteryl esters. Most of the cholesterol in Leydig cells is esterified. Treatment with LH results in hydrolysis of cholesterol esters, and free cholesterol is used for steroidogenesis. These approaches allowed us to demonstrate significant influences of OA on processes that may reduce the availability of cholesterol for testosterone synthesis in Leydig cells.

The important new findings of this study are that OA inhibits testosterone synthesis in Leydig cells independently of effects on cholesterol concentrations in the cells and inhibits hydrolysis of cholesteryl esters. OA does not cause depletion of cholesterol from Leydig cells, but appears to reduce the movement of cholesterol from Leydig cells into the incubation medium.

Loading Leydig cells with cholesterol by treating them with low concentrations of OA and LDL cholesterol had no influence on reversing the inhibitory effects of OA on testosterone synthesis. As shown in this study, OA also reduces production of testosterone and hydrolysis of cholesteryl esters[34,35] in response to LH independently of its effects on Leydig cell concentrations of cholesterol. Cholesteryl esters are hydrolyzed and used for steroid formation in the gonads and adrenal cortices. Our current observations are consistent with an effect of OA on decreasing cholesteryl esterase activity in cytosol and mitochondria, which could blunt substrate availability in the mitochondria. We cannot exclude the possibility that OA may inhibit the transport of cholesterol to the inner mitochondrial

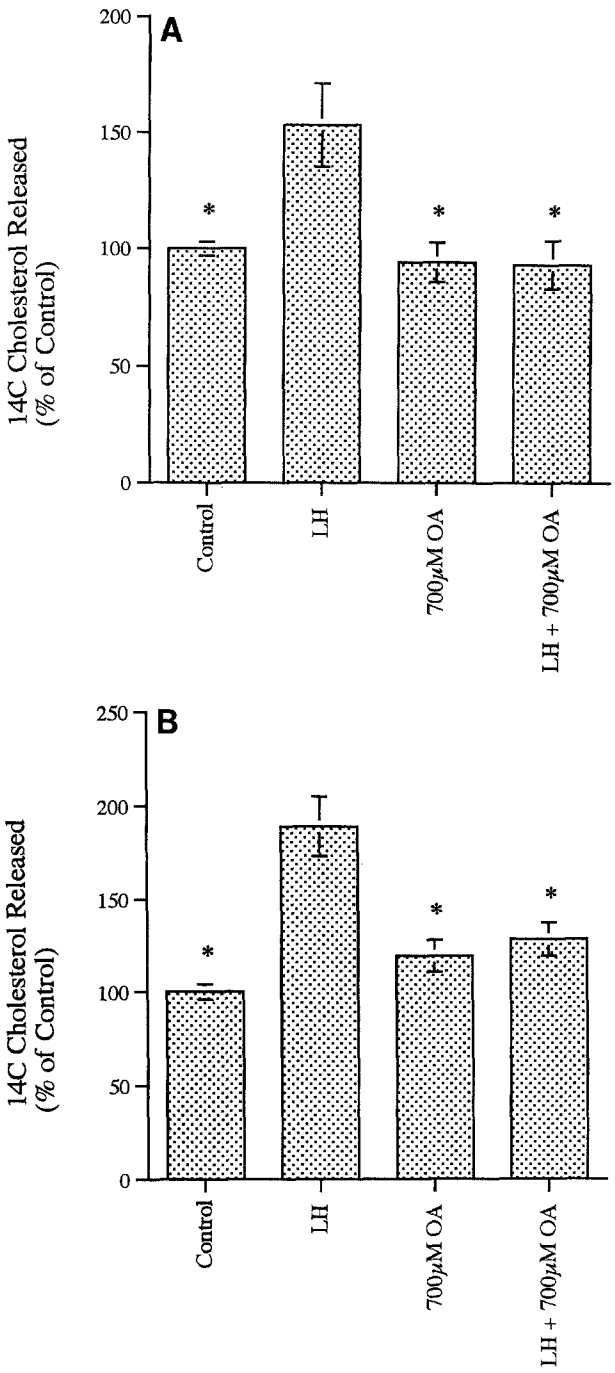

Fig 5. Effect of a 12-hour treatment with OA (700 μmol/L) on hydrolysis of cholesterol oleate in response to LH (10 ng/mL) in cytosol (A) and mitochondria (B) of isolated mouse Leydig cells. Values are the mean ± SE; n = 6. Results are expressed as a percent of the control. *$P < .025$, LH *v* other treatments.

membrane, where it can be converted to pregnenolone by the cholesterol side-chain cleavage enzyme.[1-5,36] Further studies are needed to assess these possibilities.

These results suggest that the primary effect of OA on Leydig cells is a suppression of LH-stimulated hydrolysis of cholesterol esters used for steroidogenesis. These findings agree with previous findings that cholesterol-transport inhibitors decrease steroidogenesis in cultured MA-10 Leydig tumor cells.[22,37] They also agree with observations reported by Daumerie et al,[38] in which dietary OA increased the cholesterol pool of liver cells, presumably by modulating the activity of acyl–coenzyme A cholesterol acyltransferase.

Our aim was not to investigate fully the influence of lipoprotein treatment on Leydig cell function independently of the effects of OA. High concentrations of LDL may have increased testosterone synthesis, as reported in a Leydig cell tumor[30] and in pig Leydig cells.[31] Although LDL treatment elevates Leydig cell cholesterol, these stores require the cholesterol-mobilizing effects of LH to make it available to mitochondria for initiating the testosterone synthesis pathway.

Further studies are required to determine precise biologic mechanisms by which NEFA and lipoproteins regulate Leydig cell steroidogenesis and affect cholesterol hydrolysis. These regulatory mechanisms could account for observations on the important effects of diet on plasma sex steroid levels in men. Diet is known to affect plasma lipoprotein and NEFA concentrations and could indirectly influence steroidogenesis in Leydig cells.

REFERENCES

1. Dufau ML: Endocrine regulation and communicating functions of the Leydig cell. Annu Rev Physiol 50:483-508, 1988
2. Saez J: Leydig cells: Endocrine, paracrine, and autocrine regulation. Endocr Rev 15:574-626, 1994
3. Hall PF: Testicular steroid synthesis: Organization and regulation, in Knobil E, Neill J (eds): The Physiology of Reproduction. New York, NY, Raven, 1988, pp 975-998
4. Freeman DA: Cyclic AMP mediated modification of cholesterol traffic in Leydig tumor cells. J Biol Chem 262:13061-13068, 1987
5. Hou AW, Collins DC, Schleicher RL: Sources of cholesterol for testosterone biosynthesis in murine Leydig cells. Endocrinology 127:2047-2055, 1990
6. Meikle AW, Benson SJ, Liu X, et al: Nonesterified fatty acids modulate steroidogenesis in mouse Leydig cells. Am J Physiol 257:E937-E942, 1989
7. Meikle AW, Liu X, Stringham JD: Extracellular calcium and luteinizing hormone effects on cholesterol utilization for testosterone production in mouse Leydig cells. J Androl 12:148-151, 1991
8. Goodfriend TL, Ball DL, Elliott ME, et al: Fatty acids are potential endogenous regulators of aldosterone synthesis. Endocrinology 128:2511-2519, 1991
9. Meikle AW, Stringham JD, Bishop DT, et al: Quantitating genetic and nongenetic factors influencing androgen production and clearance in men. J Clin Endocrinol Metab 67:104-109, 1988
10. Meikle AW, Bishop DT, Stringham JD, et al: Relationship between body mass index, cigarette smoking and plasma sex-steroids in normal male twins. Genet Epidemiol 6:399-412, 1989
11. Meikle AW, Stringham JD, Woodward MG, et al: Effects of a fat-containing meal on sex hormones in men. Metabolism 39:943-946, 1990
12. Hanis T, Zidek V, Sachova J, et al: Effects of dietary *trans*-fatty acids on reproductive performance of Wistar rats. Br J Nutr 61:519-529, 1989
13. Hulsmann WC: Abnormal stress reactions after feeding diets rich in (very) long-chain fatty acids: High levels of corticosterone and testosterone. Mol Cell Endocrinol 12:1-8, 1978
14. Hamalainen EK, Adlercreutz H, Puska P, et al: Decrease of serum total and free testosterone during a low-fat high-fibre diet. J Steroid Biochem 18:369-370, 1983
15. Rojdmark S: Influence of short-term fasting on the pituitary-testicular axis in normal men. Horm Res 25:140-146, 1987
16. Herbert DC: Testicular testosterone concentrations in protein-calorie malnourished rats. Arch Androl 14:45-49, 1985
17. Anderson KE, Rosner W, Khan MS, et al: Diet-hormone interactions: Protein/carbohydrate ratio alters reciprocally the plasma levels of testosterone and cortisol and their respective binding globulins in man. Life Sci 40:1761-1768, 1987
18. Longcope C, Yosha S, Young RA, et al: The effects of low protein diet and testosterone on sex hormone–binding globulin capacity in male rabbits. Metabolism 36:703-707, 1987
19. Sebokva E, Garg ML, Clandinin MY: Modulation of receptor-mediated gonadotropin action in rat testes by dietary fat. Am J Physiol 254:E708-E712, 1988
20. Reaven GM, Chen YI: Role of abnormal free fatty acid metabolism in the development of non–insulin-dependent diabetes mellitus. Am J Med 85:106-112, 1988
21. Murray DK, Nelson DH: Dexamethasone inhibition of cholesterol-stimulated glucose transport in 3T3-L1 cells. Biochim Biophys Acta 898:308-312, 1987
22. Nagy L, Freeman DA: Effect of cholesterol transport inhibitors on steroidogenesis and plasma membrane cholesterol transport in MA-10 Leydig tumor cells. Endocrinology 126:2267-2276, 1990
23. Levy H, Deane HW, Rubin BL: Visualization of steroid-3B-ol-dehydrogenase activity in tissues of intact and hypophysectomized rats. Endocrinology 65:932-943, 1959
24. Lange Y, Steck TL: Cholesterol-rich intracellular membranes: A precursor to the plasma membrane. J Biol Chem 260:15592-15597, 1985
25. Goldstein JL, Dana SE, Brown MS: Esterification of low density lipoprotein cholesterol in human fibroblasts and its absence in homozygous familial hypercholesterolemia. Proc Natl Acad Sci USA 71:4288-4292, 1974
26. Ascoli M: Effects of hypocholesterolemia and chronic hormonal stimulation on sterol and steroid metabolism in a Leydig cell tumor. J Lipid Res 22:1247-1253, 1981
27. Popplewell PY, Azhar S: Effects of aging on cholesterol content and cholesterol-mobilizing enzymes in the rat adrenal gland. Endocrinology 121:64-73, 1987
28. Liao C, Reaven E, Azhar S: Age-related decline in the steroidogenic capacity of isolated rat Leydig cells: A defect in cholesterol mobilization and processing. J Steroid Biochem 46:39-47, 1993
29. Anderson JM, Dietschy JM: Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat. J Biol Chem 253:9024-9032, 1978
30. Freeman DA, Ascoli M: Studies on the source of cholesterol used for steroid biosynthesis in cultured Leydig cells. J Biol Chem 257:14231-14238, 1982
31. Behahmed M, Reventos J, Saez JM: Steroidogenesis of

cultured purified pig Leydig cells: Effects of lipoproteins and human gonadotropin. Endocrinology 112:1952-1957, 1983

32. Quinn PG, Dombrausky LJ, Chen Y, et al: Serum lipoproteins increase testosterone production in hCG-desensitized Leydig cells. Endocrinology 109:1790-1792, 1981

33. Schumacher M, Schwarz M, Leidenberger L: Desensitization of mouse Leydig cell in vivo: Evidence for the depletion of cellular cholesterol. Biol Reprod 33:335-345, 1985

34. Iguchi T, Takasugi N, Nishimura N, et al: Correlation between mammary tumors and blood glucose, serum insulin, and free fatty acids in mice. Cancer Res 49:821-825, 1989

35. Lillioja S, Foley J, Bogardus D, et al: Free fatty acid metabolism and obesity in man: In vivo and in vitro comparisons. Metabolism 35:505-515, 1986

36. Avallet O, Vigier M, Chatelain PG, et al: Regulation by growth factors of Leydig cell differentiated functions. J Steroid Biochem Mol Biol 40:453-464, 1991

37. Gocze PM, Freeman DA: A cholesteryl ester hydrolase inhibitor blocks cholesterol translocation into the mitochondria of MA-10 Leydig tumor cells. Endocrinology 131:2972-2978, 1992

38. Daumerie CA, Woolett LA, Dietschy JM: Fatty acids regulate hepatic low density lipoprotein receptor activity through redistribution of intracellular cholesterol pools. Proc Natl Acad Sci USA 89:10797-10801, 1992