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Androgens Markedly Stimulate the Accumulation of Neutral Lipids in the Human Prostatic Adenocarcinoma Cell Line LNCaP*

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ABSTRACT
Microscopic evaluation of LNCaP cells stained with the lipophilic dye Oil red O revealed that androgens induce a marked stimulation of lipid droplet accumulation. As determined by quantitative analysis of the Oil red O extracted from the stained cells, stimulatory effects of the synthetic androgen R1881 became apparent at concentrations as low as 10^{-11} M. Maximal induction (15-fold) was reached at 10^{-8} M. Increases were observed 2 days after hormone addition and were maximal 1 day later. Accumulation of lipid droplets was also induced by mibolerone (another synthetic androgen) and by the natural androgens testosterone and dihydrotestosterone. In agreement with the aberrant ligand specificity of the mutated androgen receptor in LNCaP cells, stimulation of lipid accumulation was also apparent after treatment with progesterone and estradiol. Cortisol and the synthetic glucocorticoid dexamethasone were ineffective. The androgen antagonist Casodex (bicalutamide) abolished the stimulatory effect of R1881, further supporting the involvement of the androgen receptor. In agreement with this conclusion, no changes in lipid accumulation were observed after androgen treatment of the androgen receptor-negative prostate tumor lines PC-3 and DU-145. To investigate the nature of the lipids affected by androgens, lipid extracts were analyzed by TLC, complemented with enzymatic lipid analyses. Androgens were shown to have major effects on the content of triglycerides and cholesterol esters (33- and 7-fold stimulation, respectively), the two main classes of lipids stained by Oil red O. Phospholipid and cholesterol contents were increased by a factor of 2. Incorporation studies with [2-^{14}C]acetate revealed that androgens caused a major stimulation of 2-^{14}C incorporation into triglycerides and cholesterol esters (11- and 13-fold, respectively), suggesting that androgens act at least in part at the level of lipid synthesis. Taken together, these findings indicate that androgens, besides affecting proliferation and protein secretion, also markedly stimulate the production and accumulation of neutral lipids, revealing a novel interesting aspect of androgen regulation of LNCaP cells. (*Endocrinology* **137:** 4468–4474, 1996)

SINCE THE Nobel prize-winning findings of Huggins and Hodges more than 50 yr ago (1), androgens have been known to play a major regulatory role in the biology of human prostate cancer. Still today, most medical therapies for the treatment of proliferative disorders of the prostate are aimed at a reduction of the levels or actions of androgens. Nevertheless, the full complexity of the molecular events underlying the effects of androgens is yet to be uncovered. Of particular value in this respect is the human prostatic adenocarcinoma cell line LNCaP (2). This cell line responds to androgens with major changes in the rate of cell proliferation and the secretion of proteins such as prostate-specific antigen and has become the main experimental paradigm of androgen-responsive prostate cancer.

Recently, when searching for novel androgen-regulated genes in LNCaP cells, we isolated a gene encoding diazepam-binding inhibitor/acyl-coenzyme A (CoA)-binding protein (DBI/ACBP) (3, 4). This gene encodes a 10-kDa multifunctional polypeptide that is, for instance, involved in the regulation of various aspects of lipid metabolism. DBI/ACBP has been shown to induce the synthesis of medium chain acyl-CoA esters by goat mammary gland fatty acid synthetase (5). It is thought to bind acyl-CoA-esters (hence the name acyl-CoA-binding protein, ACBP) (5), thereby attenuating the acyl-CoA inhibition of enzymes such as acetyl-CoA carboxylase (involved in the synthesis of metabolites for fatty acid synthesis) and protecting long chain acyl-CoA esters against hydrolysis (6). In addition, DBI/ACBP was found to extract acyl-CoA from phosphatidylcholine membranes, to mediate intermembrane acyl-CoA transport, and to donate activated fatty acids for β-oxidation or triglyceride synthesis (7). Independently from the previous observations, DBI/ACBP has been shown to facilitate the translocation of cholesterol to the inner mitochondrial membrane during steroidogenesis (8).

Our finding that the expression of DBI/ACBP is controlled by androgens in LNCaP cells (3, 4) and in epithelial cells of the rat prostate (9) prompted us to investigate whether androgens, besides affecting cell proliferation and protein secretion, also influence the metabolism of lipids in LNCaP cells. Here, using simple methods, such as histochemical

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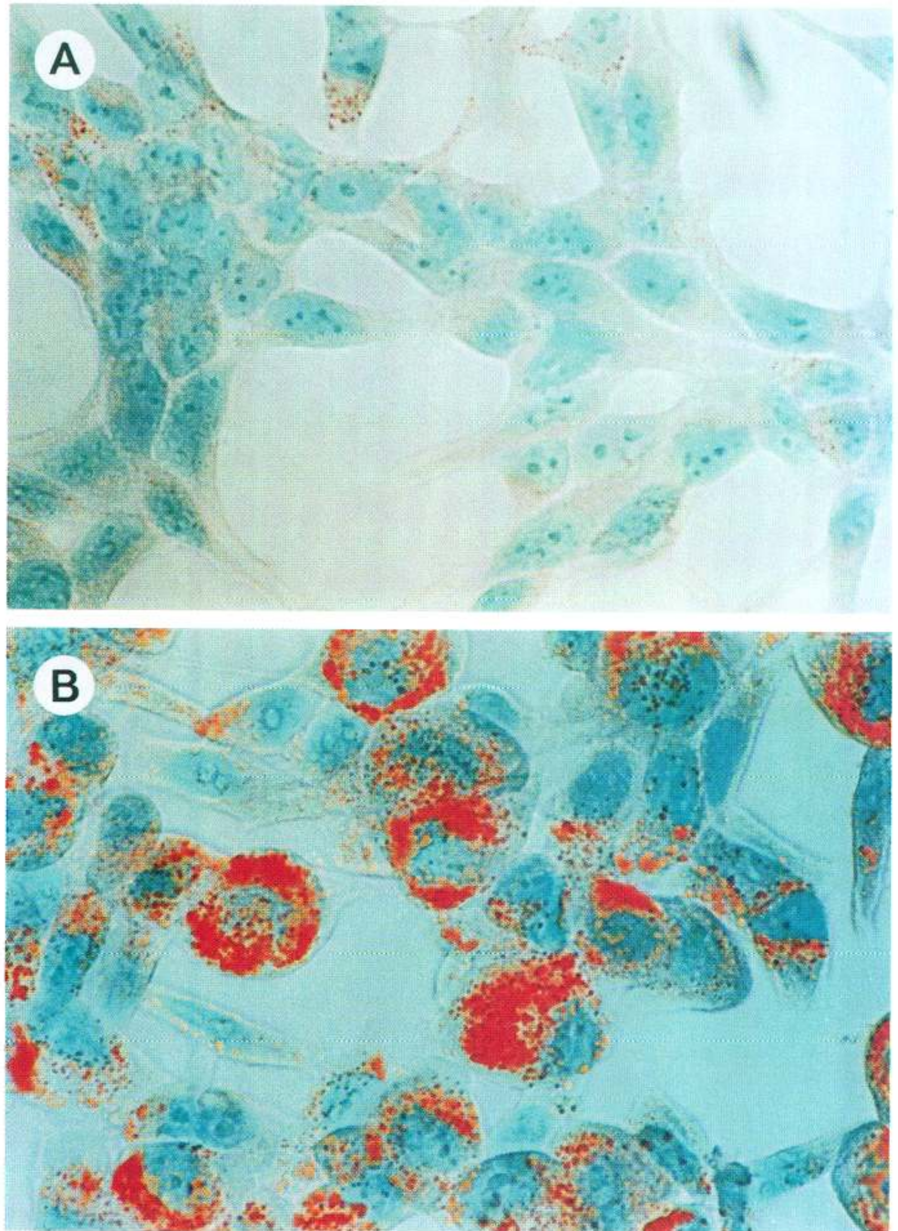

FIG. 1. Microscopic visualization of the effects of androgens on the accumulation of lipid droplets in LNCaP cells. LNCaP cells were incubated under androgen-deprived conditions (A) or in the presence of 10^{-8} M of the synthetic androgen R1881 for 4 days (B). After fixation, cells were stained with Oil red O, counterstained with methyl green, and photographed.

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staining with Oil red O, we explored the effect of androgens on the accumulation of neutral lipids in LNCaP cells.

Materials and Methods

Cell culture

The human prostatic adenocarcinoma cell line LNCaP, originally established by Horoszewics (2), was obtained from the American Type Culture Collection (Rockville, MD). Cultures were trypsinized twice weekly and were maintained in RPMI 1640 medium supplemented with 10% FCS, 3 mM L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin (Life Technologies, Gaithersburg, MD). To study the effects of steroids, cells were plated in dishes and cultured in medium containing 5% dextran-coated charcoal-stripped FCS (CT-FCS), prepared as described by Leake *et al.* (10). Steroids were dissolved in ethanol and added to the cultures. Final ethanol concentrations did not exceed 0.1%. Control cultures received similar amounts of ethanol only. Natural steroids and dexamethasone were obtained from Sigma Chemical Co. (St. Louis, MO). Mibolerone and R1881 (methyltrienolone) were purchased from DuPont-New England Nuclear (Dreiech, Germany). Casodex (bicalutamide) was kindly provided by Zeneca Pharmaceuticals (Manchester, UK) and is a trademark of Zeneca Ltd.

Oil red O staining

LNCaP cells were plated in 60-mm dishes (3×10^5 cells/dish) in medium containing 5% FCS. After 2 days, the medium was changed to RPMI-1640 with 5% CT-FCS. Cells were incubated without (control) or with hormones for 3–4 days unless otherwise indicated. Cultures were washed with PBS and fixed for 1 h in a 5% formaldehyde solution in PBS. After washing with PBS, cells were incubated for 15 min in an Oil red O solution, prepared as described by Ramirez-Zacarias *et al.* (11). For photography, cells were counterstained during 1 h with a 1% methyl green solution in 0.02 M citrate buffer, pH 5.8. For quantitative analysis, cells were plated in sextuple. Three plates were fixed and stained with Oil red O as described above. After washing with PBS, cell-bound Oil red O was extracted in 1 ml 2-propanol, and absorbance was measured at 510 nm. The absorbance of similarly treated plates, but without cells, was taken as blank and subtracted from the experimental values. The

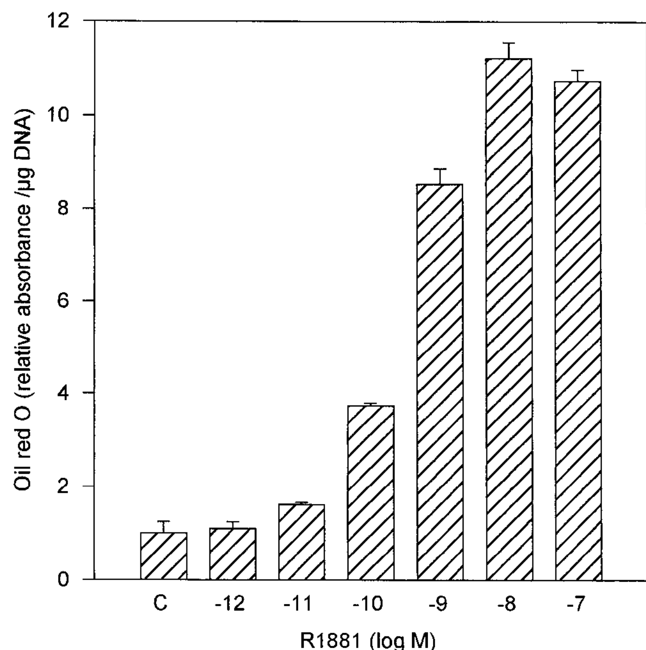

FIG. 2. Dose-dependent androgen stimulation of lipid accumulation in LNCaP cells. Cells were incubated in medium containing androgen-stripped serum and were treated with various concentrations of R1881. Control cultures (C) received ethanol vehicle only. After 4 days, cells were fixed and stained with Oil red O as described in *Material and Methods*. Cell-bound Oil red O was extracted with 2-propanol, and absorbance was measured spectrophotometrically at 510 nm. Absorbance values were corrected for differences in cellular DNA content and were expressed relative to the values of the control condition. Results shown represent the mean ± SD of incubations performed in triplicate.

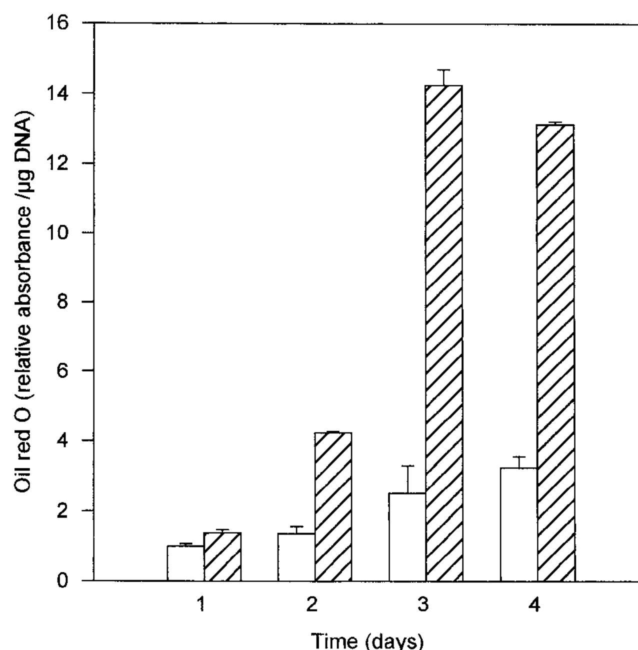

FIG. 3. Time course of the androgenic response of lipid accumulation in LNCaP cells. After incubation for 3 days in the absence of androgens, LNCaP cells were treated with ethanol vehicle (*open bars*) or 10^{-8} M R1881 (*hatched bars*). At the indicated time after treatment, cells were fixed and stained with Oil red O. Quantitation of Oil red O staining was performed spectrophotometrically after extraction of the dye with 2-propanol. Absorbance values were corrected for differences in cellular DNA content and expressed relative to the values of the control condition after 1 day. Values represent the mean ± SD of incubations performed in triplicate.

three other plates were frozen at −20 C and used to determine the DNA content using the method of Labarca and Paigen (12). Oil red O accumulation was corrected for differences in DNA content and expressed as relative absorbance, taking the control conditions (treated with ethanol) as 1.

Analysis and quantitation of cellular lipids

To study the nature of the lipids affected by androgens, LNCaP cells were plated in six 150-mm dishes at a density of 3×10^6 cells/dish and cultured for 3 days in medium containing 5% serum. Medium was changed to RPMI 1640 with 5% CT-FCS, and three dishes were treated with 10^{-8} M R1881. Four days later, the cells were washed with PBS and collected by trypsinization. Cells were resuspended in 0.8 ml PBS. Twenty microliters of this suspension were removed for determination of DNA content. The remaining suspension was mixed with 3 ml methanol-chloroform (2:1, vol/vol) in glass tubes with Teflon-lined caps. Cell debris was removed by centrifugation and reextracted after resuspension in 0.8 ml water. Organic extracts were combined, phase separated, and washed, as previously described (13). Aliquots of the lipid extracts and appropriate lipid standards were spotted onto silica gel G plates (Merck, Darmstadt, Germany). To separate neutral lipids, plates were developed in hexane-diethyl ether-acetic acid (70:30:1, vol/vol/vol). Development in chloroform-methanol-acetic acid (65:25:10, vol/vol) permitted the separation of phospholipids. Lipids were visualized with general stains (iodine vapor and molybdatophosphoric acid) and/or specific sprays (ninhydrin and ammonium molybdate) (14).

Phospholipids were determined on aliquots of the lipid extracts or scraped TLC spots, as described previously (13). The quantitation of total and free cholesterol present in the lipid extracts was based on the use of cholesterol oxidase in the presence or absence of cholesterol esterase and coupling of the produced hydrogen peroxide to the formation of the fluorescent dimer of homovanillic acid by means of peroxidase (15) (Van Veldhoven, P. P., and G. P. Mannaerts, to be published). Total glycerides, present in lipid extracts, and mono-, di-, and triglycerides were determined as follows using a commercial serum triglyceride kit from Sigma Chemical Co. After TLC separation, the corresponding spots were scraped, transferred to glass tubes and extracted with 2 ml of chloroform-methanol (1:1, vol/vol). After a centrifugation step to pellet the silica, an aliquot of the supernatant (containing up to 50 nmol glycerides) or aliquots of total lipid extracts or of a standardized triolein solution were transferred to another set of tubes and mixed with 20 µl 20% (wt/vol) Thesit (polyoxyethylene-9-lauryl ether dissolved in chloroform) before drying. The dried oily residues were dissolved in 50 µl water, followed by the addition of 450 µl reconstituted triglyceride reagent (Sigma). Reaction mixtures were incubated for 20 min at 30 C and read at 540 nm. Readings were corrected by appropriate blanks. Despite the presence of surface active compounds in the Sigma reagent, the kit, which was intended to measure serum triglycerides, did not work on dried standards or lipid extracts. Fortifying the extracts or standards with Thesit before drying allowed subsequent action of the lipase on the formed mixed micelles to proceed smoothly and to completion. In our hands, mono-, di-, and triglycerides were completely hydrolyzed to glycerol (unpublished data).

Incorporation of [2-^{14}C]acetate into lipids

LNCaP cells were plated in six-well plates (3×10^5 cells/well) in medium containing 5% CT-FCS. After 3 days, medium was changed, and three wells of each plate received R1881 at a final concentration of 10^{-8} M. The other wells were treated with ethanol vehicle. Two days later, medium was changed again, and fresh hormone was added. [2-^{14}C]Acetate (57 mCi/mmol; 1 µCi/well; Amersham International, Aylesbury, UK) was added to all wells, and incubation was continued for 24 h. Cells were washed with PBS and trypsinized. Lipids were extracted as described above, and the radioactivity of aliquots was measured by scintillation counting. [2-^{14}C]Acetate incorporation into specific lipids was visualized after separation of lipids by TLC and autoradiography. The

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more prominent lipids were scraped. Incorporation of radioactive label was quantitated by scintillation counting. Independent six-well plates were used to measure the DNA content.

Results

Effects of steroids on the accumulation of lipid droplets in LNCaP cells

To evaluate the effect of androgens on the accumulation of lipids, LNCaP cells grown in the presence or absence of androgens were fixed and stained with Oil red O, a dye that is soluble in lipids and suitable for the histochemical staining of neutral lipids such as triglycerides and cholesterol esters (11, 16). In a first series of experiments, the synthetic androgen R1881 was chosen because, unlike the natural androgen 5α-dihydrotestosterone, it is metabolically stable. As observed microscopically, LNCaP cultures maintained in the presence of 10^{-8} M R1881 for 4 days displayed a marked accumulation of Oil red O-positive lipid droplets compared to androgen-deprived cultures (Fig. 1). Accumulation of lipid droplets was heterogeneous, with some cells staining more intensely than others. In the presence of androgens, not only was the percentage of Oil red O-positive cells increased, but the number of lipid droplets per cell was also dramatically higher than that in control cells. These effects were accompanied by a major change in the morphology of the cells. Cells exposed to R1881 were overall bigger in size and round in appearance (Fig. 1). Spectrophotometric determination of Oil red O extracted from cells treated with different concentrations of R1881 and for different periods of time allowed a more detailed and quantitative analysis of the observed effects of R1881. A significant stimulation of Oil red O accumulation was apparent at R1881 concentrations as low as 10^{-11} M (Fig. 2). At a concentration of 10^{-10} M, the concentration that optimally stimulates proliferation of the cells (17), R1881 induced a 3- to 4-fold increase. Higher concentrations of R1881 further increased the accumulation of lipids, with a maximal stimulation of 7- to 15- fold at 10^{-8} M, a concentration that maximally stimulates differentiated function such as the secretion of prostate-specific antigen (17). At these concentrations, lipid accumulation became apparent 2 days after the addition of the hormone and reached a maximum 1 day later (Fig. 3). Accumulation of Oil red O was also induced by mibolerone, another synthetic androgen, and by the natural androgens testosterone and 5α-dihydrotestosterone (Fig. 4). Cells exposed to progesterone or estradiol also displayed increased Oil red O staining. Cortisol and dexamethasone were without effect. The observed steroid specificity is typical for androgen-regulated functions in LNCaP cells and is explained by the mutation in the androgen receptor in these cells, resulting in increased affinity of the androgen receptor for progestins and estrogens (18). The involvement of the androgen receptor is further evidenced by inhibition of the effects of R1881 by the androgen antagonist Casodex. In analogy with the inhibition of R1881-stimulated cell proliferation (Esquenet, M., unpublished observations), 10^{-5} M Casodex was used to efficiently block the effects of 10^{-10} M R1881 (Fig. 5). At these concentrations, Casodex reduced R1881-stimulated lipid accumulation to levels similar to those with Casodex treatment alone. No Oil red O staining was observed in the androgen receptor-negative prostate tumor cell lines DU-145 and PC-3, and in these lines androgen treatment did not induce measurable lipid accumulation.

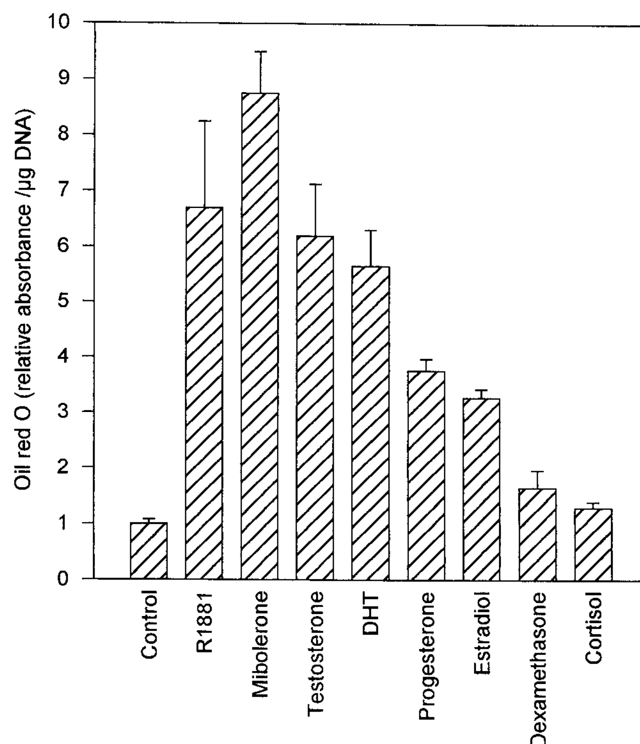

FIG. 4. Steroid specificity of lipid accumulation in LNCaP cells. LNCaP cells were incubated in charcoal-stripped medium supplemented with ethanol vehicle (Control) or with 10^{-8} M of the indicated steroids. Four days after the treatment, cells were fixed and stained with Oil red O. Cell-bound Oil red O was extracted with 2-propanol and quantitated spectrophotometrically. Absorbance values were corrected for differences in cellular DNA content and were expressed relative to the values of the control condition (no steroids, vehicle only). Results shown represent the mean ± SD of incubations performed in triplicate. DHT, 5α-Dihydrotestosterone.

Identification and quantification of lipids affected by androgens

To investigate the nature of the lipids affected by androgens, lipid extracts from LNCaP cells grown in the presence or absence of 10^{-8} M R1881 during 4 days were analyzed by TLC. In agreement with our results obtained by staining with Oil red O, marked changes were observed in the amount of neutral glycerolipids and cholesterol esters (Fig. 6). The contents of phospholipids and cholesterol were barely changed. To quantify the effects of androgens, the cellular levels of specific lipids were determined. As Table 1 illustrates, the contents of triglycerides and cholesterol esters expressed per μg DNA were increased 33- and 7-fold, respectively, after exposure to R1881. Also, mono- and diglyceride levels tended to be elevated, but exact quantitation was not possible because the levels in untreated controls were near the limit of detection. In agreement with the observed change in morphology of the cells, androgens also caused a slight (2-fold) stimulation of phospholipid content.

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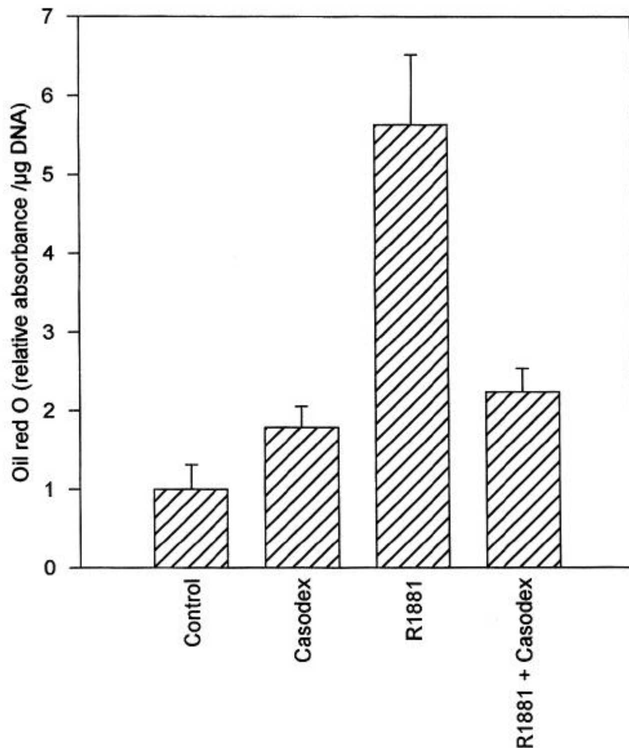

FIG. 5. Effects of the antiandrogen Casodex on the androgen-induced accumulation of lipids in LNCaP cells. LNCaP cells in androgen-deprived medium were treated with ethanol vehicle (Control), 10^{-5} M Casodex, 10^{-10} M R1881, or the combination of 10^{-5} M Casodex and 10^{-10} M R1881. After 3 days, cells were fixed and stained with Oil red O. Absorbance values of the Oil red O extracted from the cells were corrected for differences in cellular DNA content and expressed relative to the values under control conditions. The results shown represent the mean ± SD of incubations performed in triplicate.

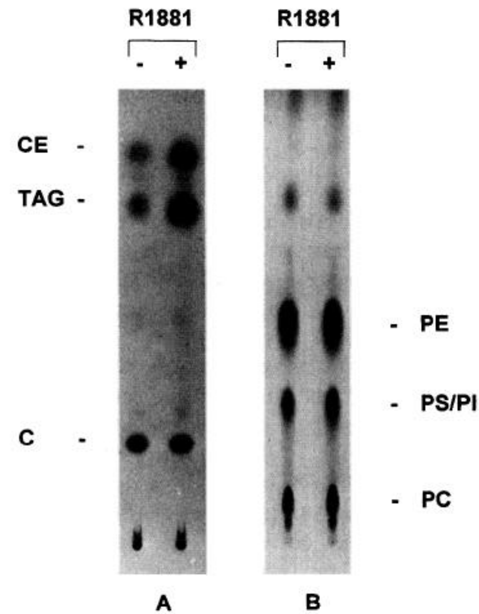

FIG. 6. Identification of lipids affected by androgens. After 4 days of incubation in medium containing androgen-deprived serum supplemented with ethanol vehicle (−) or 10^{-8} M R1881 (+), LNCaP cells were trypsinized, and lipids were extracted as described in *Materials and Methods*. Equal aliquots of the lipid extracts were analyzed by TLC for neutral lipids (A) and phospholipids (B). Lipids were revealed by molybdatophosphoric acid spray. The migration of standards is indicated. CE, Cholesterol ester; TAG, triacylglycerol; C, cholesterol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; PC, phosphatidylcholine.

TABLE 1. Effects of androgens on the accumulation of various classes of lipids

Fraction	nmol/µg DNA		Fold stimulation
	Control	R1881	
Phospholipids	2.266 ± 0.116	5.268 ± 0.692	2.3
Cholesterol	0.582 ± 0.024	1.204 ± 0.154	2.1
Cholesterol esters	0.072 ± 0.001	0.520 ± 0.030	7.2
Triacylglycerol	0.046 ± 0.001	1.530 ± 0.274	33.3

LNCaP cells, cultured in 150-mm dishes, were treated with 10^{-8} M R1881 or ethanol vehicle (control). Four days later, cells were collected by trypsinization, and lipids were extracted. Phospholipids, cholesterol, cholesterol esters, triacylglycerols, and cellular DNA were measured as described in *Materials and Methods*. Values represent the mean ± SD of incubations performed in triplicate.

Effects of androgens on de novo synthesis of lipids in LNCaP cells

To explore the possibility that the increased accumulation of triacylglycerol and cholesterol esters was due to androgen-induced changes in the *de novo* synthesis, LNCaP cells exposed to 10^{-8} M R1881 for 2 days were incubated with [2-^{14}C]acetate for 24 h. Lipid extracts were prepared, and incorporation of labeled acetate was measured by scintillation counting. As Table 2 shows, overall [2-^{14}C]acetate uptake and incorporation into lipids were increased 2-fold after androgen treatment. Specific incorporation into various classes of lipids was determined by scintillation counting of lipid spots separated by TLC. R1881 treatment induced an 11- and a 13-fold stimulation of ^{14}C incorporation in triglycerides and cholesterol esters, respectively (Table 2). Effects on phospholipids were only moderate (1.8-fold).

Discussion

Making use of Oil red O staining and quantitative enzymatic lipid determinations, we demonstrated that androgens, besides affecting cell proliferation and protein secretion, cause a marked (up to 15-fold) stimulation of the accumulation of neutral lipids in the human prostatic adenocarcinoma cell line LNCaP. Incorporation studies with labeled acetate suggest that these effects are at least in part related to androgen-induced increases in lipid synthesis.

Several lines of evidence indicate that the observed effects of androgens are mediated by the androgen receptor. 1) Accumulation of lipid droplets, as observed microscopically after staining with Oil red O and as measured quantitatively after extraction of Oil red O, was noticed after treatment of the cells with synthetic agonists (R1881 and mibolerone) as well as with natural androgens (testosterone and dihydrotestosterone). 2) The observed steroid specificity reflected the specificity of the mutated androgen receptor in LNCaP cells. 3) The effects of androgens could be neutralized by the antiandrogen Casodex. 4) No androgen-induced effects on lipid accumulation were observed in the androgen receptor-negative prostate cell lines PC-3 and DU-145.

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TABLE 2. Effect of androgens on the uptake and incorporation of [2-^{14}C]acetate into lipids

Fraction	dpm × 10^{-3}		Fold increase
	Control	R1881	
Total cellular lipids	507 ± 26	1137 ± 14	2.2
Phospholipids	220 ± 9	408 ± 39	1.8
Cholesterol	16 ± 1	51 ± 3	3.2
Triglycerides	12 ± 1	132 ± 11	10.8
Cholesterol esters	3 ± 2	38 ± 4	12.7
Total secreted lipids	79 ± 6	52 ± 5	−1.5

LNCaP cells grown in six-well plates were treated with R1881 at a final concentration of 10^{-8} M or with ethanol vehicle (control). Two days later, medium was changed again, and fresh hormone was added together with [2-^{14}C]acetate (57 mCi/mmol; 1 μCi/well). After 24 h, cells were trypsinized, and lipids were extracted. The radioactivity of aliquots was measured by scintillation counting. [2-^{14}C]acetate incorporation into specific lipids was determined after separation of lipids by TLC and scraping of lipid spots into scintillation fluid. Results shown represent the mean ± SD of incubations performed in triplicate. DNA content, as measured in independent plates, was similar in both conditions.

The nature of the accumulating lipids was analyzed by TLC and enzymatic lipid determination. In agreement with the staining specificity of Oil red O, these techniques revealed that the major classes of lipids affected by androgens were triglycerides (a 33-fold increase) and cholesterol esters (a 7-fold increase), typical neutral storage products found in lipid droplets. Incorporation studies with labeled acetate demonstrated that LNCaP cells have the capacity to synthesize these lipids *de novo*. The finding that the incorporation of labeled acetate is stimulated by androgens strongly suggests that the observed increase in neutral lipid accumulation is at least in part the result of stimulatory effects on enzymes or regulatory proteins involved in lipogenesis. Preliminary experiments indeed suggest that androgens stimulate the expression and activity of fatty acid synthase, one of the key enzymes involved in lipogenesis (Swinnen, J. V., unpublished observations). Interestingly, using antisera recognizing fatty acid synthase, this enzyme has been shown to be highly expressed in selected prostate cancers, and its use as a potential progression marker has been proposed (19, 20). As mentioned previously, we recently reported that androgens up-regulate the expression of DBI/ACBP, a multifunctional polypeptide involved in the regulation of several aspects of lipid metabolism. Whether the observed changes in DBI/ACBP may contribute to the androgenic effects seen on lipid accumulation in LNCaP cells remains to be investigated. Also, additional effects at the level of lipid uptake and/or turnover cannot be excluded at the present time.

Whether the observed androgen-induced accumulation of neutral lipid droplets is a peculiarity of LNCaP cells or whether this phenomenon may be generalized to other normal or cancerous prostatic epithelial cells remains to be explored. In this respect it is worth mentioning that the normally differentiated human prostate secretes not only proteins and citrate, but also significant amounts of various lipids (21, 22). In agreement with this differentiated function is the finding that the accumulation of lipid droplets in LNCaP cells follows a dose-response curve very similar to that of the accumulation of prostate-specific antigen in the medium, suggesting that in LNCaP cells, lipid accumulation reflects a differentiated state. In support of this hypothesis is the finding that no lipid droplets were observed in the poorly differentiated PC-3 and DU-145 cells. Interestingly, results similar to those reported here for LNCaP cells have been documented for the human breast cancer cell line T47D in response to progesterone (and, to a lesser extent, to androgens) (23), revealing a novel parallel between breast and prostate cancer cells. Evidence for the secretion of newly synthesized lipids has been found in both cell lines. However, hormone treatment tended to decrease rather than stimulate the secretion of lipids. Apart from a possible role in lipid secretion, lipid synthesis and accumulation may be related to energy metabolism. In this context, stored lipids might be used as source of energy for energy-demanding processes such as fluid and protein secretion. Alternatively, accumulation of lipid droplets might represent a means by which prostate tumor cells divert energy otherwise lost by the secretion of citrate.

Taken together, the finding that androgens markedly stimulate the production and accumulation of lipids in LNCaP cells uncovers a novel and interesting aspect of the androgen regulation of these cells and provides a sensitive parameter of androgen action that can be followed using fast, inexpensive, and simple methods.

Acknowledgment

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References

1. **Huggins C, Hodges CV** 1941 Studies on prostatic cancer: effect of castration, of estrogen, and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1:293–297
2. **Horoszewicz JS, Leong S, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP** 1983 LNCaP model of human prostatic carcinoma. Cancer Res 43:1809–1818
3. **Swinnen JV, Esquenet M, Heyns W, Rombauts W, Verhoeven G** 1994 Androgen regulation of the messenger RNA encoding diazepam-binding inhibitor/acyl-CoA-binding protein in the human prostatic adenocarcinoma cell line LNCaP. Mol Cell Endocrinol 104:153–162
4. **Swinnen JV, Esquenet M, Rosseels J, Claessens F, Rombauts W, Heyns W, Verhoeven G** 1996 A human gene encoding diazepam-binding inhibitor/acyl-CoA-binding protein: transcription and hormonal regulation in the androgen-sensitive human prostatic adenocarcinoma cell line LNCaP. DNA Cell Biol 15:197–208
5. **Mogensen IB, Schulenberg H, Hansen HO, Spencer F, Knudsen J** 1987 A novel acyl-CoA-binding protein from bovine liver. Biochem J 241:189–192
6. **Rasmussen JT, Rosendal J, Knudsen J** 1993 Interaction of acyl-CoA binding protein (ACBP) on processes for which acyl-CoA is a substrate, product or inhibitor. Biochem J 292:907–913
7. **Rasmussen JT, Faergeman NJ, Kristiansen K, Knudsen J** 1994 Acyl-CoA-binding protein (ACBP) can mediate intermembrane acyl-CoA transport and donate acyl-CoA for β-oxidation and glycerolipid synthesis. Biochem J 299:165–170
8. **Papadopoulos V, Brown AS** 1995 Role of the peripheral-type benzodiazepine receptor and the polypeptide diazepam binding inhibitor in steroidogenesis. J Steroid Biochem Mol Biol 53:103–110
9. **Swinnen JV, Vercaeren I, Esquenet M, Heyns W, Verhoeven G** 1996 Androgen regulation of the messenger RNA encoding diazepam-binding inhibitor/acyl-CoA-binding protein in the rat. Mol Cell Endocrinol 118:65–70
10. **Leake RE, Freshney RI, Munir I** 1987 Steroid response *in vivo* and *in vitro*. In: Green B, Leake RE (eds) Steroid Hormones: A Practical Approach. IRL Press, Washington DC, pp 213–214
11. **Ramirez-Zacarias JL, Castro-Munozledo F, Kuri-Harcuch** 1992 Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry 97:493–497
12. **Labarca C, Paigen K** 1980 A simple, rapid, and sensitive DNA assay procedure. Anal Biochem 102:344–352
13. **Van Veldhoven P, Bell RM** 1988 Effect of harvesting methods, growth con-

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ditions and growth phase on diacylglycerol levels in cultured human adherent cells. Biochim Biophys Acta 959:185–196
14. **Kates M** 1986 Techniques of lipidology. In: Burdon RH, van Knippenberg PH (eds) Laboratory Techniques in Biochemistry and Molecular Biology, ed 2, vol 3, part 2. Elsevier, Amsterdam, pp 186–278
15. **Van Veldhoven PP** 1995 Activity measurements of acyl-CoA oxidases in human liver. J Inherited Metab Dis [Suppl 1] 18:125–134
16. **Pearse AGE** 1968 Histochemistry: Theoretical and Applied, ed 3. Churchill, London, vol 1:398–446
17. **Esquenet M, Swinnen JV, Heyns W, Verhoeven G** 1996 The control of LNCaP cell proliferation and differentiation: actions and interactions of androgens, 1α,25-dihydroxycholecalciferol, all-*trans* retinoic acid, 9-*cis* retinoic acid and phenylacetate. Prostate 28:182–194
18. **Veldscholte J, Berrevoets CA, Ris-Stalpers C, Kuiper GGJM, Jenster G, Trapman J, Brinkmann AO, Mulder E** 1992 The androgen receptor in LNCaP cells contains a mutation in the ligand binding domain which affects steroid binding characteristics and response to antiandrogens. J Steroid Biochem Mol Biol 41:665–669
19. **Shurbaji MS, Kuhajda FP, Pasternack GR, Thurmond TS** 1992 Expression of oncogenic antigen 519 (OA-519) in prostate cancer is a potential prognostic indicator. Am J Clin Pathol 97:686–691
20. **Epstein JI, Carmichael M, Partin AW** 1995 OA-519 (fatty acid synthase) as an independent predictor of pathologic stage in adenocarcinoma of the prostate. Urology 45:81–86
21. **Mann T, Lutwak-Mann C** 1981 Male Reproductive Function and Semen. Springer-Verlag, Berlin pp 306–308
22. **Vignon F, Clavert A, Koll-Back MH, Reville P** 1992 On the glandular origin of seminal plasma lipids in man. Andrologia 24:341–343
23. **Chambon M, Rochefort H, Vial H, Chalbos D** 1989 Progestins and androgens stimulate lipid accumulation in T47D breast cancer cells via their own receptors. J Steroid Biochem 33:915–922

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