Molecular and Cellular Biochemistry **145:** 1–9, 1995.

Regulatory effect of various steroid hormones on the incorporation and metabolism of [^{14}C]stearate in rat hepatoma cells in culture

Carlos Alberto Marra* and María J. T. de Alaniz*

Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, Facultad de Ciencias Médicas, 60 y 120, 1900 La Plata, Argentina

Received 20 July 1994; accepted 21 November 1994

Abstract

We have examined the incorporation and metabolism of [^{14}C] stearic acid within the total lipids of HTC rat-hepatoma cells in suspension culture in presence and in absence of steroidal hormone stimulation. Both, glucocorticoids (dexamethasone, cortisol and corticosterone) and mineralocorticoids (deoxycorticosterone and aldosterone) as well as the estrogen β-estradiol and the androgen testosterone enhanced the extent of Δ9 desaturation to oleic acid of the saturated precursors, whereas only the two mineralocorticoids affected the incorporation rate of the exogenous acid into total cellular lipids, thus promoting a little stimulation. Furthermore, all the hormones tested increased the radiolabelling of the total cellular phospholipids except deoxycorticosterone and testosterone, the former having no effect and the latter exerting a moderate inhibition. On the other hand, the incorporation of ^{14}C into neutral lipids was stimulated by testosterone, in contrast to the inhibition of this parameter observed exclusively with either the mineralocorticoids or the estrogen. Within the phospholipid subclasses, the radiolabelling of phosphatidylcholine was augmented by means of all the steroids tested save deoxycorticosterone and testosterone, whereas phosphatidylethanolamine exhibited a decrease only in the presence of testosterone. In a similar fashion, within the neutral lipids, the predominating triglyceride fraction was preferentially labelled – at the expense of other subclasses of lesser abundance – upon treatment with the steroids except aldosterone, which exerted no effect. The results obtained were correlated with those changes observed in the mass distribution of the different lipid subclasses either with or without prior hormonal stimulation. (Mol Cell Biochem **145:** 1–9, 1995)

Key words: rat-hepatoma, steroidal hormones, lipid metabolism

Abbreviations: ALD – aldosterone; ANOVAR – analyses of variance; CE – cholesterol esters; Cl – cortisol; CLN – cardiolipin; Cst – corticosterone; CH – cholesterol; DG – diacylglycerides; DOC – 11-deoxycorticosterone; Dx – dexamethasone phosphate salt; βE – 17-β-estradiol; FAME – fatty acid methyl esters; GLC – gas-lipid chromatography; HEPES – N-2-hydroxyethyl-piperazine-N-2-ethanosulfonic acid; HTC – hepatoma tissue culture; IMEM-Zo – improved minimal essential medium-Zinc option; LPC – lysophosphatidyl-choline; LPL – lysophospholipids; MG – monoacylglycerides; NEFA – free fatty acids; PC – phosphatidyl-choline; PE – phosphatidyl-ethanolamine; PG – phosphatidyl-glycerol; PI – phosphadyl-inositol; PS – phosphadidyl-serine; SM – sphingomyelin; TG – triacylglycerides; TLC – thin-layer chromatography; Tst – testosterone

Introduction

It is well known that palmitic and stearic acids are desaturated at the Δ9 position (probably by two independent enzymes) to give palmitoleic and oleic acids, respectively [1]. Each one of these monounsaturated acids then becomes the initial pre-

*Members of the Carrera del Investigador Científico, CONICET, Argentina.
Address for offprints: C.A. Marra, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), CONICET-UNLP, Facultad de Cincias Médicas, 60 y 120, 1900 La Plata, Argentina

cursor for the biosynthesis of an independent family of polyunsaturates [2, 3]. The Δ9 desaturase may also be considered as a lipogenic enzyme, whose function is to desaturase dietary or newly synthesized saturated fatty acids for deposition primarily in triacylglycerides [1, 4, 5]. Other studies have indicated that palmitoyl- and stearoyl-CoA desaturases play a relevant role in providing monoenoic acids for export as lipoprotein components [6, 7]. These families of desaturating enzymes also contribute to maintaining the appropriate fluidity in the phospholipids of biological membranes which structures in turn, regulate a wide variety of membrane-associated events [6, 8–10].

The desaturation rate at Δ9 position would also seem to be highly dependent on the hormonal state of the animal. In microsomal preparations of rat or avian livers Δ9 desaturase activity is significantly stimulated by insulin [1, 5, 11] or thyroxin [1, 11, 12] administration. Moreover, the effect of the natural estrogen 17-β-estradiol was investigated by Lippiello and co-workers in rooster liver [6] and by Kasturi and Joshi in the murine 3T3-L1 cell line [5]. These researchers have reported that the biosynthesis of monoenoic fatty acids was stimulated by estradiol supplementation. In addition, we have also reported that Δ9 desaturase was strongly stimulated by a soluble protein induced by dexamethasone [13] or 11-deoxycorticosterone [14] treatment. By contrast the effect of other steroid hormones on this desaturase enzyme has not been investigated in mammals. The study of the hormonal regulation of Δ9 desaturase activity in whole animal is often complicated by the occurrence of indirect changes in the concentration of hormones and other biological molecules in blood. The use of isolated cells maintained in a chemically defined culture medium, i.e. without serum, would thus prove of fundamental importance in the system under investigation in order to determine the primary physiological effect of different steroid hormones on the regulation of either lipid metabolism in general or stearoyl-CoA desaturase activity in particular. Of further advantage was the fact that the HTC-cell system used for these studies responds to physiological concentrations of various steroids of different chemical structures and diverse biological activities.

Materials and methods

Chemicals

[1–^{14}C]Stearic acid (58.9 mCi/mmol, 99% pure) was from New England Nuclear Corp., Boston, MA, USA. Unlabelled stearic and eicosa-11-enoic acids were provided by Nu-Chek Prep, Elysian, MN, USA. The acids were stored in benzene under an atmosphere of nitrogen at 20°C. Concentrations and purities were confirmed by both liquid-scintillation counting and the gas-liquid chromatography (GLC) of fatty acid methyl esters prepared in the presence of appropriate internal standards. Neutral lipids and phospholipids standards for thin-layer chromatography (TLC) were obtained from Serdary Research Laboratory Inc., London, Ontario, Canada. Fatty-acid methyl-ester mixtures for GLC identifications, defatted bovine serum albumin and HEPES (N-2-hydroxyethyl-piperazine-N-2-ethanosulfonic acid) were from Sigma Chemical Company, St. Louis, MO, USA. Dexamethasone (sodium phosphate salt) was from Merck, Sharp and Dhome, Buenos Aires, Argentina. Other steroid hormones used were obtained from Sigma Chemical Company. All chromatographic solvents were RPE grade and obtained from Carlo Erba, Milan, Italy. Other chemicals used were reagent grade and provided by commercial sources.

Cell culture

The hepatoma cells used in these experiments were from the Morris minimal deviation hepatoma number 7288c, a solid hepatoma from which the ascites-tumor line (HTC cells) had been derived [15]. HTC cells were maintained and grown *in vitro* at 37°C in surface culture on Swim's 77 (S-77) medium supplemented with 10% (v/v) calf serum, under conventional sterile conditions as previously described [16].

Assay procedure

Monolayers of HTC cells in the logarithmic growth phase (approximately 72 h) were detached from the growing surface with 0.1% (w/v) trypsin [17]. They were then suspended in modified IMEM-Zo medium [18] in which the $CaCl_2$ concentration was reduced to 1.5 mM, linoleic acid was omitted and HEPES was added to 5-mM final concentration. Soybean trypsin inhibitor, type I (0.1%, w/v) was then added during the following washing steps. The cell suspension was sedimented twice by centrifuging in 20 ml of modified IMEM-Zo medium at 4–5°C for 10 min (500 × g). The final pellet was resuspended in the same medium and counted in a hemocytometer. Aliquots (5×10^6 cells) were finally incubated at 37°C under an atmosphere of 95% oxygen and 5%, (v/v) carbon dioxide in a metabolic shaker (70 rpm). The incubation volume was 5 ml in 25 ml siliconized Erlenmeyer flasks. Cell viability (> 96%) was assessed by the criterion of trypan-blue dye exclusion [19]. Two types of experiments were designed in order to study the effect of various steroid hormones on the incorporation and desaturation of stearic acid as well as the distribution of the endogenously generates oleate among the different lipid subclasses. In these experiments, the cells were incubated for 12 h in the presence of dexamethasone (Dx), cortisol (Cl), corticosterone (Cst),

11-deoxycorticosterone (DOC), aldosterone (ALD), 17-β-estradiol (βE) or testosterone (Tst), at 1.0 μM concentration. Three hours before the end of the incubation period, the media were supplemented with a mixture of labelled and unlabelled stearic acid 80 μM (0.5 μCi/flask). The acid was added as the sodium salt bound to defatted bovine serum albumin, according to Spector *et al.* [20] in a ratio of 6 nmol fatty acid to 1 nmol albumin. One set of control cells was supplemented with delipidated albumin at the same concentration as the fatty-acid-supplemented group; while another group of controls was treated identically, but with the omission of hormones. These latter flasks were, however, supplemented with the vehicle in which the hormones were dissolved (ethanol at 20 mM). In all instances, at the end of the incubation period, the cell suspensions were transferred to ice-cold tubes and centrifuged at 500 × g for 5 min. Each pellet was washed three times with 5 ml of cold 0.85% (w/v) NaCl and resuspended in the same solution. An aliquot of the cell suspension was used to determine cell viability and the amount of cellular protein [21]. The rest was centrifuged at 2,000 × g for 10 min, the supernatant was discarded, and each cell pellet was processed separately for lipid analysis.

Cellular-lipid extraction and separation

Lipids were extracted from the cell pellets by means of the method of Folch *et al.* [22]. The organic phases, containing more than 97% of the total radioactivity, were further separated by two chromatographic procedures. In the first system, the neutral lipids were separated by TLC on 0.25 mm thick silica gel G-60 plates (heat-activated) in a solvent system containing hexane:diethylether:acetic acid (80:20:1, v:v:v) [23]. Under these conditions, the phospholipids remained at the origin. The individual neutral lipids separated in this run were identified by comparison with a standard mixture containing (Rf values in parenthesis) monoacyl-glycerides (MG) (0.10), diacylglycerides (DG) (0.20), cholesterol (CH) (0.37), free fatty acids (NEFA) (0.40), triacylglycerides (TG) (0.72), fatty-acid methyl esters (FAME) (0.81), and cholesterol esters (CE) (0.90), which pure species were applied to each plate. Phospholipids were separated in a second TLC system [24] by two successive developments in the same direction. The first mobile phase was chloroform:methanol:ammonium hydroxide:water (70:25:3.5:1.5, v:v:v:v) while the second one was chloroform:methanol:acetic acid:water (80:10:2:0.75, v:v:v:v). The different subclasses of phospholipids were identified by reference to the following standard mixture developed in parallel with the samples: lysophosphatidyl choline (LPC) (<0.13), phosphatidylserine (PS) (0.19), phosphatidylinositol (PI) (0.25), sphingomyelin (SM) (0.30), phosphatidylcholine (PC) (0.39), phosphatidylethanolamine (PE) (0.55), cardiolipin (CLN) (0.69), and phosphatidyl-glycerol (PG) (0.71).

Lipid analysis

The plates were firstly developed and then dried under nitrogen. Lanes containing samples were covered with a glass plate, while the lipid markers were briefly sprayed with a tincture of iodine 1.5% (w:v) in methanol. Areas of interest were scraped off the plates and isolated lipids recovered by elution with chloroform/methanol, (1:1, v:v). Once dried, the samples were dissolved in 500 μl of cold chloroform/methanol, and aliquots were taken for liquid-scintillation counting in a Wallac 1214 Rackbeta liquid scintillation counter (Pharmacia, Turku, Finland) (97% efficiency for ^{14}C), interfaced to an Olivetti M-240 computer system. The plates were also scanned for radioactivity detection (radio-TLC) by means of a TLC-Proportional Radioactivity Scanner (Berthold LB-2832, Wildbad, Germany) equipped with a Hewlett-Packard 3396-A Data Station. No significant differences were found between radioactivity quantification by TLC-scanning or by liquid-scintillation counting. Some plates were charred [25] and quantified densitometrically (Karl Zeiss, Germany) according to the method of Katz *et al.* [26]. Aliquots of the original lipid extracts were dried and subjected to trans-esterification by heating in 2.5 ml 4 N HCl/methanol at 68°C for 3 h under nitrogen. The resulting FAME were extracted three times with 2 ml of hexane. Organic phases from each tube were pooled and evaporated under nitrogen. The residue were prepared for GLC analysis and dissolved in 20–30 μl of hexane containing 1 μg/tube of eicosa-11-monoenoic acid as an internal standard. The GLC of FAME was performed in an Acromat GC-100 apparatus (Redhill, Surrey, England) with a 6 ft glass column packed with 10% SP-2330 on 100–200 mesh Chromosorb WAW-DMCS (Supelco Inc, Bellefonte PA), USA. After 4 min initial hold, the oven was programmed from 140–220°C at 1.5°C/min. The distribution of radioactivity among the FAME was determined by means of a Packard 894 Gas-Flow Proportional Counter (Downers Grove, Ill, USA) interfaced to the chromatograph through a nominal 10:1 splitter. A linear dual-pen recorder (LKB-2210 Two-Channel Recorder, Bromma, Sweden) was used to obtain simultaneously radioactivity and mass tracings for each chromatographic separation. The individual FAME were identified by comparison of their relative retention times with authentic standards, and the mass distribution was calculated electronically by quantification of the peak areas.

All results were expressed as the means and standard error calculated from 3–5 independent analyses. Paired or unpaired student's *t* test and 'ANOVAR' were used for statistical evaluations. $p < 0.01$ was considered significantly different when compared to the corresponding control group.

Results and discussion

We have firstly examined the effect of physiological concentrations of various steroidal hormones on the uptake and Δ9 desaturation of [^{14}C]stearic acid in suspensions of HTC cells (Fig. 1). Although the effect of Tst was not statistically significant, this androgen exhibited a marked tendency to inhibit the uptake of the exogenous acid. Furthermore, from all the hormones tested in this system, only DOC and ALD increased the uptake of labelled stearic acid in a significant fashion (Fig. 1, *solid bars*). Such increment in the incorporation of this exogenous metabolic precursor could be explained by an increase in its subsequent utilization. Mineralocorticoids have been reported to produce a general activation of lipid metabolism, and ALD, in particular, was shown to increase the degradation of specific phospholipid fatty acids [27, 28] and to stimulate oleic-acid metabolism [29] as well as phospholipase A_2 activity [30] in urinary bladder. These effects, occurring within 30 min of the beginning of the hormone treatment and persisting for up to 6 h, lead to a depletion in the intracellular level of stearic acid [29]. By contrast Tst caused a decrease in the level of sterified fatty acids in human aortic smooth-muscle cells along with a reciprocal increment in their free-fatty acid content [31]. If the androgen acts in a similar fashion in our system, the resulting major availability of free fatty acids might well explain the decreased uptake of exogenous stearic acid that we observed (Fig. 1).

Despite several papers about the stimulation of lipid metabolism by glucocorticoids, specifically an increase in the biosynthesis of phosphatidylcholine [32, 33] and triacylclycerides [34], the uptake of labelled stearate into cellular lipids was not significantly modified under Dx, Cl or Cst treatments in our studies (Fig. 1). In view of these findings, we decided to investigate the effect of glucocorticoids on the incorporation of [^{14}C]18:0 into neutral or polar lipid subclasses; the results obtained will be discussed below.

All the hormones tested evoked a significant increase in cellular Δ9 desaturation (Fig. 1, *hollow bars*) in contrast to the results obtained for the fatty acid uptake. The increase in oleic acid formation upon exposure of the HTC cells to hormones was 50% with glucocorticoids, 65% with Tst or mineralocorticoids, and *ca.* 100% with βE with respect to control values. A similar stimulation of Δ9 desaturase activity was previously found in our laboratory with liver microsomal preparations from rats injected with physiological doses of Tst [35] or Dx phosphate [13]. Moreover, González Rodríguez and co-workers demonstrated that the activity of the liver microsomal Δ9 desaturase was markedly enhanced after the injection of βE to ovariectomized rats [36]. Lipiello *et al.* [6] reported that the same estrogen, when administered to roosters, significantly increased the activity of the enzyme with stearic acid as substrate. The stimulatory effects caused

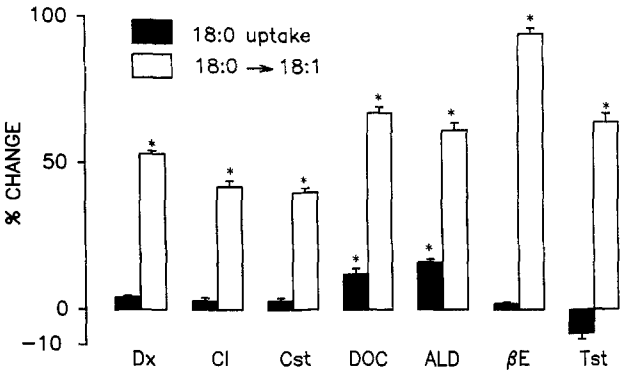

Fig. 1. Effect of the treatment of HTC cells with various steroid hormones on the uptake (■) and Δ9 desaturation (●) of stearic acid. Aliquots of HTC cells suspended in IMEM-Zo medium (1. 10^6 cells/ml) were incubated for 12 h at 37°C in the presence or absence of different steroid hormones at a final concentration of 1 μM. During the last 3 h of incubation a mixture of lablled and unlabelled sodium stearate (80 μM, 0.50 μCi/flask) was added to all flasks. At the end of the experiment, the cells were centrifuged (10 min at 500 × g) and washed by a second sedimentation. The pellets were extracted by the method of Folch *et al.*, and the lipid extracts were processed to determine the uptake of ^{14}C and Δ9 desaturation of the substrate. For details see *Materials and methods* section. The results were expressed as the percent change with respect to the mean value for the two controls: 40.0 ± 0.7 nmol of stearic acid incorporated per mg of cellular protein and 33.2 ± 0.7 nmol of oleic acid produced per mg of cellular protein. Each value was the mean of 4–5 independent determinations. Vertical lines represent 1 SEM. Asterisks indicate values significantly different from controls at $p < 0.01$.

by the other steroidal hormones studied in the present experiments (Fig. 1) have not been previously reported.

These changes in desaturase activity observed in the presence of gluco- or mineralocorticoids would involve modifications in the fatty acid profiles of target tissues. Indeed, Lien *et al.* reported that ALD administration to toads simultaneously decreased the content of saturates and increased the levels of monoenoates in their urinary bladders [29]. We also observed similar alterations in the hepatic microsomal lipids obtained from rats treated with either ALD or DOC [37] as well as in the total lipids of HTC cells incubated with Dx phosphate [38]. In the present experiments, we also found that all the steroidal hormones studied were able to produce the same analytical changes in the saturated/monoenoic fatty acid ratio. All the hormones tested also modified the fatty acid profiles which were characterized by a *ca.* 40% increase in the amount of monounsaturates over control values (Data not shown).

The marked changes evoked by the steroidal hormones on the uptake and metabolism of the stearic acid and the important modifications in the fatty acid composition prompted us to examine the distribution of the labelled substrate into polar and neutral lipids (Fig. 2) and to characterize its distribution among the main lipid subclasses (Fig. 3). Accordingly, our study on the incorporation of [^{14}C]stearate into polar lipids of HTC cells treated with various steroids demonstrated that

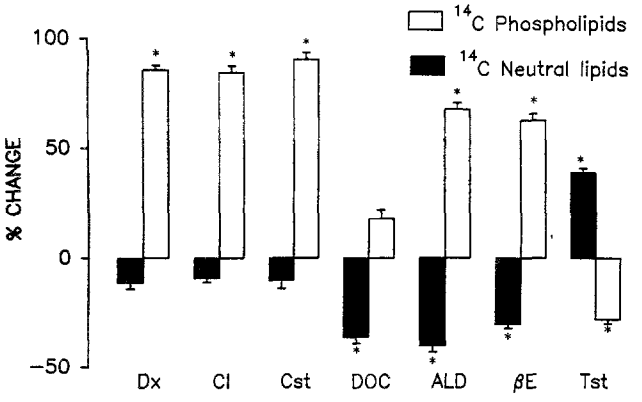

Fig. 2. Effect of the treatment of HTC cells with various steroid hormones on the incorporation of [^{14}C]stearic acid into neutral lipids (■) or phospholipids (□). Suspensions of HTC cells were incubated under conditions similar to those described in Fig. 1. At the end of the experiment, the cells were centrifuged and washed. Cellular lipids were extracted by the method of Folch *et al.* Phospholipids and neutral lipids were separated by TLC. The plates were scanned for radioactivity detection; and then the lipids were scraped off the plates and reextracted in chloroform:methanol (1:1, v:v) mixture. Aliquots of these extracts were taken for liquid-scintillation counting. For details see *Materials and methods*. Each value was the mean of 4 independent analyses and is expressed as the per cent change with respect to the corresponding control: 855.10^3 and 420.10^3 dpm/mg of cellular protein for the uptake of total radioactivity into phospholipids and neutral lipids, respectively. Vertical lines represent 1 SEM.

all the hormones tested caused an increase in the incorporation of label ranging from 46–91% above control levels with the exception of DOC, which produced not significant changes, and Tst, which inhibited the uptake of [^{14}C]stearate by about 30% (Fig. 2). With respect to neutral lipids, the glucocorticoid hormones (Dx, Cl or Cst) produced no significant modifications in the incorporation of labelled substrates (stearic acid and/or its direct metabolite oleic acid), while the mineralocorticoids, DOC or ALD, decreased the uptake of this radioactivity by ca. 40%. The effect caused by 17-b-estradiol was opposite to that of testosterone, the androgen being the only steroidal species produces an enhancement of label in the HTC-cell phospholipids. Thus, while estrogen inhibited the uptake of ^{14}C by approximately 30%, Tst produced a 40% increase in the incorporation over the control data. Hence, the effect of testosterone was of particular interest because that hormone exhibited an action opposite to that seen with the others. In those cells exposed to testosterone, the ratio of ^{14}C incorporation into neutral vs. polar lipid fractions was 2 to 14-fold higher than the values obtained with any of the other steroid hormones studied.

The results shown in Fig. 2 could suggest that glucocorticoid hormones produce an increase in phospholipid biosynthesis with minor changes in the metabolism of neutral lipids. Since changes in the incorporation of ^{14}C into one particular lipid subclass upon hormonal treatment could be offset by variations in another one, it is important to consider the data

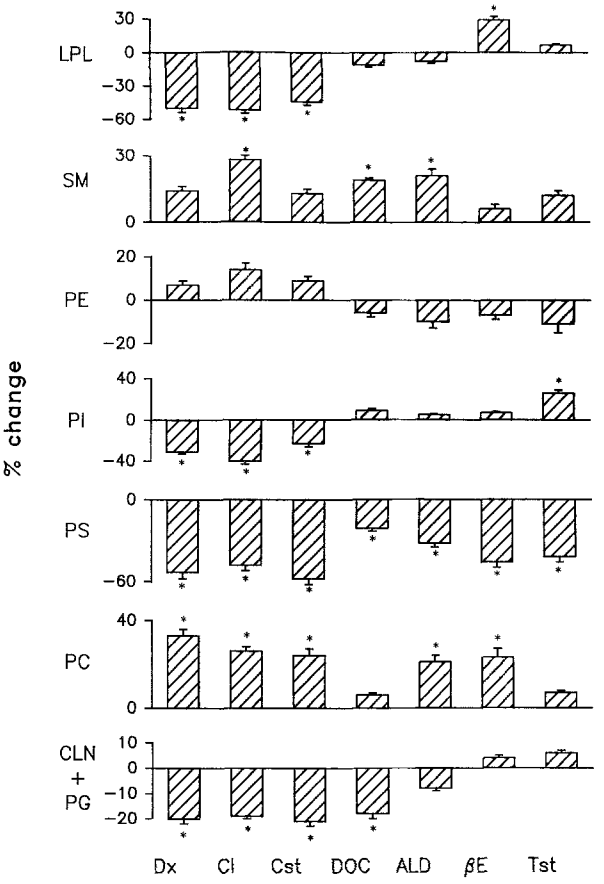

Fig. 3. Effect of steroid hormones on the incorporation of [^{14}C]stearic acid into different cellular lipids. Aliquots containing 5.10^6 HTC cells suspended in IMEM-Zo medium, were incubated and harvested as described in the legend to Fig. 1. The lipids from each pellet were then extracted by the method of Folch *et al.*, and the lipid subclasses were separated by TLC as stated in the text. The plates were next scanned for radioactivity detection and finally sprayed with iodine solution to reveal the position of each lipid species. After calculation of the respective *Rf* values, the spots were scraped off the plates and prepared for liquid-scintillation counting. Results were expressed as the per cent change with respect to the corresponding control values (all expressed as dpm.10^3/mg of cellular protein, with the lipid subclasses in parenthesis): 314.6 (PE), 244.5 (PC), 42.7 (CLN+PG), 106.8 (NEFA), 243.6 (TG), and 52.5 (CE). Any lipid subclasses, not included in the figure, failed to show significant changes with respect to the controls. Each value is the mean of 3–4 independent determinations. Vertical lines represent 1 SEM.

reported in Fig. 3. In these experiments we investigated the distribution of ^{14}C within the principal lipid subfractions in HTC cells incubated for 3 h in the presence of [^{14}C]stearic acid (80 μM) after a 12 h treatment with steroid hormone at a concentration of 1.0 μM. Glucocorticoids were found to produce an enhancement on ^{14}C incorporation into the PC fraction, with only minor changes occurring in the other polar lipid subclasses. A possible explanation for this fact are the following observations: (a) Glucocorticoids stimulated pulmonary PC biosynthesis [32, 33] as well as the activity of three enzymes related to phospholipid synthesis: glycero-

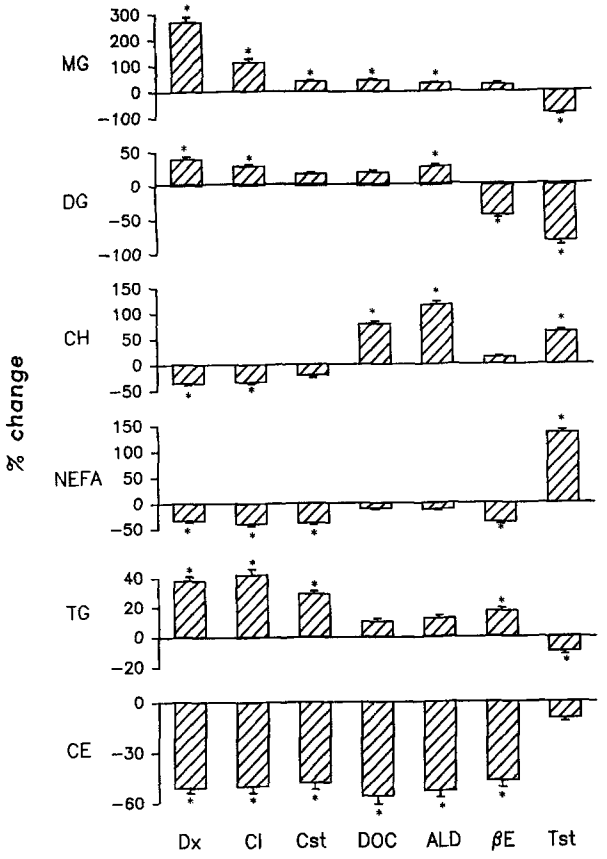

Fig. 4. Effect of steroidal hormones on the per cent weight distribution of phosphorylated lipid subclasses from HTC cells. Suspensions of the hepatoma cells were incubated under the conditions described in the legend to Fig. 3 except that the mixture of labelled and unlabelled stearic acid was replaced by unlabelled sodium stearate at a final concentration of 80 μM. After separation of the phospholipids by TLC, the plates were charred and quantified densitometrically as described in *Materials and methods*. Each datum is the mean of 3–4 independent determinations and is expressed as the per cent change for the experimental group in question respect to the corresponding control densitometric values, with the latter being expressed as the per cent of the sum of the optical densities for all the lipid subclasses. The control data used for reference in this way are the following (lipid fraction in parenthesis): 4.0 (LPL), 8.1 (SM), 36.8 (PE), 10.5 (PI), 7.4 (PS), 28.6 (PC), and 4.6 (CLN+PG). Vertical lines represent 1 SEM.

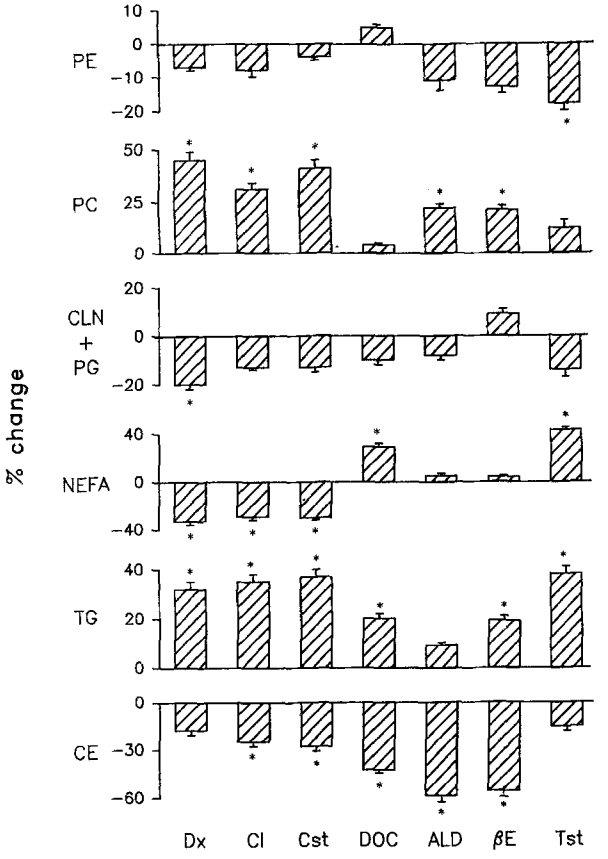

Fig. 5. Effect of the steroidal hormones on the per cent weight distribution of nonphosphorylated lipid subclasses from HTC cells. Suspensions of the hepatoma cells were incubated under the conditions described in the legend to Fig. 3 except that the mixture of labelled and unlabelled stearic acid was replaced by unlabelled sodium stearate at a final concentration of 80 μM. After separation of the neutral lipids by TLC, the plates were charred and quantified densitometrically as described in *Materials and methods*. Each datum is the mean of 3–4 independent determinations and is expressed as the per cent change for the experimental group in question respect to the corresponding control densitometric values, with the latter being expressed as the per cent of the sum of the optical densities for all the lipid subclasses. The control data used for reference in this way are the following (lipid fraction in parenthesis): 0.1 (MG), 2.5 (DG), 22.2 (CH), 7.1 (NEFA), 57.6 (TG), and 10.5 (CE). Vertical lines represent 1 SEM.

phosphate acyltransferase [39], phosphatidate phosphotransferase [33, 39], and glycerol-phosphate dehydrogenase [40]. (b) Corticosteroids also induced fatty acid synthetase in fetal lung, the products from which pathway, either as free acid or incorporated into phospholipids, activates cholinephosphate cytidyltransferase [41]. By contrast, the synthetic Cl analogue, Dx, caused a small decrease (*ca.* 20%) in the labelling of the CLN + PG fraction (Fig. 3). Moreover, in a similar fashion to glucocorticoids, though in a lesser extent, ALD and βE also stimulated the incorporation of ^{14}C into the PC subclass. With βE, this increment is likely to be the consequence of the stimulation of two independent enzymic activities, both leading to an increased overall capacity for PC synthesis: cholinephosphate cytidyltransferase [33] and glycerol kinase [43].

The comparison of the results shown in Figs 2 and 3 reveals a clear correlation between the effects of the gluco- and mineralocorticoids studied on the incorporation of ^{14}C into total phospholipids and into the PC subclass. Moreover the inhibition observed in the labelling of polar lipids in the presence of Tst (Fig. 2) could be adequately accounted for by the decreased capacity of the CLN + PG and PE subclasses to incorporate and/or metabolize stearic acid (Fig. 3), since PE is one of the principal fractions within the phospholipids of HTC cells [44, 45].

Regarding neutral lipids, glucocorticoids caused a significant decrease in the incorporation of labelled stearate into NEFA and CE (save in the latter subclass where inhibition in the presence of Dx failed to reach a significant level). These negative effects (Fig. 3) were, however, almost completely counterbalanced by a simultaneous stimulation of ^{14}C incorporation into TG (Fig. 3), with the end result of no significant change in the neutral lipid fraction (Fig. 2). These findings are consistent with the strong stimulation by glucocorticoids of TG biosynthesis observed in isolated hepatocytes [34]. Accordingly, the substantial increment in the labelling of the TG fraction (Fig. 3) would indicate an increased secretion of triacylglycerols in the form of particles having the properties of very low density lipoproteins [34]. The endogenous mechanism by which this particular class of steroids alters the rate of TG biosynthesis and/or secretion in ours and in other systems is, however, still unknown.

Mineralocorticoids also produced significant alterations in the neutral lipids of HTC cells (Fig. 3). DOC inhibited the incorporation of [^{14}C]stearate into CE and stimulated the incorporation of labelled metabolites into TG and NEFA. Exposure to ALD also inhibited significantly the uptake of ^{14}C into CE without any change in the other neutral lipid subclasses. To the best of our present knowledge, these observations represent the first experimental evidence that mineralocorticoids markedly modify the neutral lipid metabolism.

Sexual steroids also caused changes in the neutral lipids of HTC cells. The uptake of ^{14}C into TG was stimulated by both Tst of βE treatment. The androgen produced an increase in the incorporation of ^{14}C into NEFA, while the estrogen decreased the total radioactivity associated with CE. Previous reports had indicated that estrogens increased significantly the biosynthesis of TG in liver [46]. We found a similar behavior in HTC cells treated with βE. Exposure of aortic smooth-muscle cells to estrogens produced a simultaneous inhibition of hydroximethylglutaryl-CoA reductase activity with a concomitant stimulation of the incorporation of exogenous cholesterol. These effects when combined produce an elevation in cellular CE levels [31], which enhancement is offset by Tst and thoroughly reversed by tamoxifen treatment [31, 46, 47]. Furthermore, earlier experiments reported by Nassen *et al.*, had demonstrated that estrogen treatment also produced a marked decrease in the rate of cellular-cholesterol esterification [31]. These results were also obtained in our experimental system (Fig. 3).

The main parameter influencing the uptake and subsequent metabolic utilization of an exogenous fatty acid is the lipid subclass into which that precursor and its eventual metabolites are incorporated. Accordingly, the quantification of the different lipids in HTC cells, with and without hormonal treatment, is necessary for a meaningful interpretation of all experimental results presented above. For this reason, Fig. 4 shows the relative mass distribution of polar lipids in HTC cells in the presence and absence of treatment with different steroidal hormones. Similarly, Fig. 5 presents the corresponding results for neutral lipids in the cells.

As stated above, glucocorticoids stimulate the biosynthesis of PC in lung [32–34, 39–41]. We could speculate that this effect also occurs in HTC cells as shown in Fig. 3. Moreover, this supposition is supported by the increment in the relative amount of PC from those data in Fig. 4. The significant decrease observed in the percentage of PI under glucocorticoid treatment (Fig. 4) does not correlate with any other similar change in the uptake of ^{14}C into this lipid subclass (data not shown). A parallel behavior was exhibited by the PS fraction. The decreases observed in the relative amounts of CLN + PG fraction under Dx, Cl or Cst treatments were of similar magnitude, but only in the case of Dx the results here were correlated well with the decreased incorporation of ^{14}C seen in this lipid fraction (Fig. 3).

Mineralocorticoids produced a significant increase in the percentage of SM and a concomitant decrease in the relative content of PS (Fig. 4). Unfortunately, these lipid fractions showed a very poor capacity to incorporate labelled substrates so that no differences were found respect to control cells (data not shown). Thus, it is not possible to draw any comparison between the results from the experiments in Figs 3 and 4 for the SM or PS fractions. The hormonal effects of mineralocorticoids on these lipid fractions are of interest since they have not been previously described. The decreases produced by DOC over the relative amounts of the CLN + PG and PS subclasses would be evoked through the reported 'glucocorticoid-like' effect displayed by this hormone (but not by ALD) on hepatic tissue [47]. ALD, on the other hand, caused a significant increase in the percentage of PC, effect not seen with DOC (Fig. 4). Moreover, this increment correlates well with the uptake of radioactivity registered in this lipid subfraction after the 3 h labelling with [^{14}C]stearate (Fig. 3). The biological effect of ALD over PC metabolism was entirely novel. Notwithstanding, previous studies carried out on amphibians, have indicated that this mineralocorticoid hormone increases the phospholipase A_2 activity and stimulates the acylation-deacylation cycle in the urinary bladder of the toad [27, 28]. Despite these results, we failed to detect any significant change in the level of the LPL fraction after ALD treatment (Fig. 4).

βE and Tst produced a marked inhibition in the proportion of PS (Fig. 4). The treatment with βE increased the percentage of PC and LPL, while the incubation in the presence of Tst caused an increment in the relative amount of PI. These results are in agreement with previous studies from other laboratories. For example, βE was reported to stimulate significantly the biosynthesis of PC in lung and the activity of cholinephosphate-cytidyltransferase [33]. Experiments carried out with muscle cells have also demonstrated that estrogen produces an anabolic effect on phospholipid me-

tabolism [31]; whereas androgens either antagonize estrogenic action or produce no changes at all [31, 33].

Glucocorticoids injected to rats have been reported to stimulate the biosynthesis of TG and to produce fat liver [34]. In this work we have observed not only a stimulation in the uptake of labelled stearic acid into TG, (Fig. 3) but also a significant increase in the relative mass distribution of TG, DG and MG (Fig. 5). Hence, these stimulatory effects would be synergized by oleic acid derived from stearate by Δ9 desaturation since previous reports have indicated that oleate can significantly stimulate the activity of diacylglycerol-transferase in isolated hepatocytes [39]. Figure 5 also shows that incubation in the presence of glucocorticoids resulted in a decrease in the amount of CH and CE with the effect of Cst being of lesser importance. On the other hand, all the glucocorticoids tested evoked a similar reduction in the content of NEFA (Fig. 5), effect that well correlated with the diminished capacity for ^{14}C uptake exhibited by this lipid fraction (Fig. 3).

Both mineralocorticoids, DOC and ALD, caused an increment in the level of the metabolic precursors of TG, (i.e.; MG and DG). The relative amount of TG was not, however, significantly modified. Furthermore, both steroid hormones increased the content of CH while they simultaneously decreased the level of CE (Fig. 5). A comparison of the results shown in Figs 3 and 5, reveals that the specific incorporation of ^{14}C in these latter lipid fractions was not markedly decreased under DOC or ALD treatment.

The relative contents of NEFA, DG, and CE were reduced after incubating the cells in the presence of βE. This steroid also produced an increment in the percentage abundance of TG, effect that is in agreement with those results previously obtained in aortic smooth-muscle cells [31]. In the latter cell type, however, the estrogen caused elevations in NEFA and CE, which changes were not observed with the HTC cells (Fig. 5). An adequate explanation for these discrepancies would no doubt be based on the elucidation of differences in other aspects of the total lipid metabolism of these two cell populations and must thus remain unresolved until such a time as those pertinent data became available for the aortic cells.

With Tst treatment, the relative abundance of both MG and DG decreased significantly, whereas the cellular TG content remained unchanged. This finding indicates that the principal pathway for the TG biosynthesis is depressed and that the metabolic precursors for the alternative biosynthetic process, MG and DG, are accordingly actively recruited to maintain the endogenous pool of TG at its normal physiologic levels. Figure 2 shows that Tst treatment produces the highest ratio of ^{14}C incorporation into neutral lipids *vs.* phospholipids, which result could be derived from the observed marked increase in the cellular NEFA content (*ca.* 140%), which effect would sequester ^{14}C labelling within the neutral-lipid fraction.

In conclusion, these experiments have demonstrated for the first time the effects produced by a wide variety of steroidal hormones on the incorporation and metabolism of stearic acid. It was also shown that Δ9 microsomal desaturation is strongly stimulated and that the incorporation of labelled metabolites into different lipid fractions is modified by hormonal treatment. We would anticipate that the information derived from these studies will be useful in elucidating the mechanism underlying other metabolic changes arising from the effect of steroidal hormones, such as the regulation of water permeability by mineralocorticoids [27, 28], the action of glucocorticoids on the characteristics of the air-alveolar barrier [32, 33], the differences in lipid metabolism between males and females as attributed to sexual-hormone balance [48], and related actions involving alleviations of chronic diseases by means of the administration of natural or synthetic steroids.

Acknowledgements

This study was supported by grants from CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Argentina. The authors are grateful to Elsa Claverie for her technical assistance.

References

1. Sprecher H: The mechanism of fatty acid chain elongation and desaturation in animals. In: J.D.G. Kramer, F.D. Sower and W.J. Pidgeon (eds). High and low erucic acid rafessed oil. Academic Press, New York, pp 385–411, 1983
2. Brenner RR: The mechanism step in the animal biosynthesis of polyunsaturated fatty acids. Lipids 6: 567–575, 1971
3. Brenner RR: The oxidative desaturation of unsaturated fatty acids in animals. Mol Cell Biochem 3: 41–52, 1974
4. Volpe JJ, Vagelos PR: Mechanisms and regulation of biosynthesis of saturated fatty acids. Physiol Rev 56: 339–317, 1976
5. Kasturi R, Joshi VC: Hormonal regulation of stearoyl coenzyme A desaturase activity and lipogenesis during adipose conversion of 3T3-L1 cells. J Biol Chem 257: 12224–12230, 1982
6. Lipiello PM, Holloway CT, Gardfield SA, Holloway PW: The effects of estradiol on stearoyl-CoA desaturase activity and microsomal membrane properties in rooster liver. J Biol Chem 254: 2004–2009, 1979
7. Lamb RG, Bow SJ, Wright TO: Effects of chronic insulin and glucagon exposure on the biosynthesis of glycerolipids by cultured hepatocytes. J Biol Chem 257: 15022–15025, 1982
8. Jeffcoat R, James AT: Fatty acid metabolism and its regulation. In: S. Numa (ed). Elsevier, Amsterdam, Vol. 7, pp 85–112, 1984
9. Leikin AI, Brenner RR: *In vivo* cholesterol removal from liver microsomes induces changes in fatty acid desaturase activities. Biochim Biophys Acta 963: 311–319, 1988
10. Leikin AI, Brenner RR: Cholesterol-induced microsomal changes modulate desaturase activities. Biochim Biophys Acta 922: 294–303, 1987

11. Joshi VC, Aranda LP: Hormonal regulation of the terminal enzyme of microsomal stearoyl coenzyme A desaturase in avian liver explants. J Biol Chem 254: 11779–11782, 1979
12. Gómez Dumm INT de, Alaniz MJT de, Brenner RR: Effects of thyroxine on Δ6 and Δ9 desaturation activity. In: N.G. Bazan, R.R. Brenner and N. Giusto (eds). Exp Med Biol. Plenum Press, New York, pp 609–617, 1977
13. Marra CA, Alaniz MJT de, Brenner RR: A dexamethasone-induced protein stimulates Δ9 desaturase activity in rat liver microsomes. Biochim Biophys Acta 958: 93–98, 1988
14. Marra CA, Alaniz MJT de: 11-Deoxycorticosterone, an inducer of a factor stimulating Δ9 desaturase activity in liver microsomes. Acta Physiol Pharmacol Latinoam 41: 277–285, 1991
15. Thompson EB, Tomkins GM, Curran JF: Induction of tyrosine aminotransferase by steroid hormones in a newly established tissue cultured cell line. Proc Natl Acad Sci USA 56: 296–303, 1966
16. Alaniz MJT de, Ponz G, Brenner RR: Biosynthesis of unsaturated fatty acids in cultured minimal deviation hepatoma 7288c cells. Acta Physiol Latinoam 25: 1–11, 1975
17. Thompson EB, Anderson CV, Lippman ME: serum-free growth of HTC cells containing glucocorticoid- and insulin-inducible tyrosine aminotransferase and cytoplasmic glucocorticoid receptors. J Cell Physiol 86: 403–412, 1975
18. Richter A, Sanford KK, Evans VJ: Influence of oxygen and culture media on plating efficiency of some mammalian tissue cells. J Natl Cancer Inst 49: 1705–1712, 1972
19. Jauregui HO, Hayner NT, Driscoll JL, Williams-Holland R, Lipsky MH, Galletti PM: Trypan blue dye uptake and lactate dehydrogenase in adult rat hepatocytes freshly isolated cells, cell suspensions, and primary monolayer cultures. *In Vitro* 17: 1100–1110, 1981
20. Spector AA, Steinberg DS, Tanaka A: Uptake of free fatty acids by Ehrlich ascites tumor cells. J Biol Chem 240: 1032–1041, 1965
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265–275, 1951
22. Folch J, Lees M, Stanley S: A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 226: 497–503, 1957
23. Malins DC, Mangold HK: Analysis of complex lipid mixtures by thin-layer chromatography and complementary methods. J Am Oil Chem Soc 37: 297–300, 1968
24. Neskovic NM, Kostic DM: Quantitative analysis of rat liver phospholipids by a two-step thin-layer chromatography procedure. J Chromatog 35: 297–300, 1968
25. Blank ML, Sahmit JA, Privett OS: Quantitative analysis of lipids by thin-layer chromatography. J Am Oil Chem Soc 41: 371–376, 1964
26. Katz SS, Shipley G, Small DM: Physical chemistry of the lipids of human atherosclerotic lesions. J Clin Invest 58: 200–211, 1976
27. Goodman DBP, Allen JE, Rasmussen H: Studies on the mechanism of action of aldosterone: hormone-induced changes in lipid metabolism. Biochemistry 10: 3825–3831, 1971
28. Edelman IS: Mechanism of action of aldosterone: energetic and permeability factors. J Endocrinol 81: 49P–53P, 1979
29. Lien EL, Goodman DBP, Rasmussen H: Effects of an acetyl-coenzyme A carboxylase inhibitor and sodium-sparing diuretic on aldosterone-stimulated sodium transport, lipid synthesis, and phospholipid fatty acid composition in the toad urinary bladder. Biochemistry 14: 2749–2754, 1975
30. Yorio T, Bentley PJ: Phospholipase A and the mechanism of action of aldosterone. Nature 271: 79–81, 1978
31. Naseen SM, Heald FP: Sex-mediated lipid metabolism in human aortic smooth-muscle cells. Biochem Biophys Res Comm 144: 284–291, 1987
32. Duval D, Durant S, Homo-Delarche F: Non-genomic effects of steroids. Interactions of steroid molecules with membrane structures and functions. Biochim Biophys Acta 737: 409–442, 1983
33. Ballard PL: Hormonal regulation of pulmonary surfactant. Endocrinol Rev 10: 165–180, 1989
34. Mangiapane EH, Brindley DN: Effects of dexamethasone and insulin on the synthesis of triacylglycerols and phosphatidylcholine and the secretion of very-low-density lipoproteins and lysophosphatidylcholine by monolayer cultures of rat hepatocytes. Biochem J 233: 151–160, 1986
35. Marra CA, Alaniz MJT de: Influence of testosterone administration on the biosynthesis of unsaturated fatty acids in male and female rats. Lipids 24: 1014–1019, 1989
36. González Rodríguez S, Nervi AM, Peluffo RO: Effects of estradiol and environmental temperature changes on rat liver Δ6 microsomal desaturase activity. Lipids 21: 440–443, 1986
37. Marra CA, Alaniz MJT de: Mineralocorticoids modify rat liver Δ6 desaturase activity and other parameters of lipid metabolism. Biochem Int 22: 483–493, 1990
38. Marra CA, Alaniz MJT de, Brenner RR: Dexamethasone blocks arachidonate biosynthesis in isolated hepatocytes and cultured hepatoma cells. Lipids 21: 212–219, 1986
39. Pittner RA, Fears R, Brindley DN: Interactions of insulin, glucagon, and dexamethasone in controlling the activity of glycerol-phosphate acyltransferase and the activity and subcellular distribution of phosphohydrolase in cultured rat hepatocytes. Biochem J 230: 525–534, 1985
40. Meyer RD, Preston SL, McMorris FA: Glycerol-3-phosphate-dehydrogenase is induced by glucocorticoids in hepatocytes and hepatoma cells *in vitro*. J Cell Physiol 114: 203–208, 1983
41. Pope TS, Smart DA, Rooney SA: Hormonal effects on fatty-acid synthase in cultured fetal rat lung: induction by dexamethasone and inhibition of activity by triiodothyronine. Biochim Biophys Acta 959: 169–177, 1988
42. Xu ZX, Smart DA, Rooney SA: Glucocorticoid induction of fatty-acid synthetase mediates the stimulatory effect of the hormone on choline-phosphate cytidyltransferase activity in fetal rat lung. Biochim Biophys Acta 1044: 70–76, 1990
43. Pittner RA, Feras R, Brindley DN: Effects of cyclic AMP, glucocorticoids, and insulin on the activities of phosphatidate phosphohydrolase, tyrosine aminotransferase, and glycerol kinase in isolated rat hepatocytes in relation to the control of triacylglycerol synthesis and gluconeogenesis. Biochem J 225: 455–462, 1985
44. Wood R: Lipids of cultured hepatoma cells: I. Effect of serum lipid levels on cell and media lipids. Lipids 8: 690–701, 1973
45. Wood R, Falch J: Lipids of cultured hepatoma cells: II. Effect of media lipids on cellular phospholipids. Lipids 8: 702–710, 1973
46. McCune SA, Rimmell F, Hoversland RC, Jurin RR: Inhibition of cholesterol and fatty acid synthesis in rats by an oestrogen antagonist both in isolated hepatocytes and *in vivo*. Biochem Soc Trans 14: 1198, 1986
47. Shui Pang T, Archer TK, Duley RG: Biphasic effects of estrogen on apolipoprotein synthesis in human hepatoma cells: Mechanism of antagonism by testosterone. Proc Natl Acad Sci USA 83: 3111–3115, 1986
48. Alaniz MJT de, Marra CA: Glucocorticoid and mineralocorticoid hormones depress liver Δ5 desaturase activity through different mechanisms. Lipids 27: 599–604, 1992
49. Huang YS, Horrobin DF: Sex differences in n-3 and n-6 fatty acid metabolism in EFA-depleted rats. Proc Soc Exp Biol Med 185: 291–296, 1987