J Korean Med Sci 1999; 14: 277-85
ISSN 1011-8934

The Metabolic Effects of Estriol in Female Rat Liver

The effects of estriol on oxygen uptake, glucose release, lactate and pyruvate production, β-hydroxybutyrate and acetoacetate production in perfused rat liver as well as, carbon uptake in rat liver and intracellular calcium in isolated Kupffer cells were investigated. Basal oxygen consumption of perfused liver increased significantly in estriol or ethanol-treated rats. But these increased effects were blocked by gadolinium chloride pretreatment. In a metabolic study, pretreatment with estriol resulted in a decrease in glucose production and in glycolysis while an increase in ketogenesis. A more oxidized redox state of the mitochondria was indicated by increased ratios of perfusate [lactate] / [pyruvate] and decreased ratios of perfusate [β-hydroxybutyrate] / [acetoacetate]. Carbon uptake of Kupffer-cell increased significantly in estriol-treated rats. But these increased uptake were not shown in rats pre-treated by gadolinium chloride blocking phagocytosis. In isolated Kupffer cells from estriol-treated rats, intracellular calcium was more significantly increased after addition of lipopolysaccharide (LPS) than in controls. These findings suggest that the metabolic effects of estriol (two mg per 100 mg body wt) can be summarized to be highly toxic in rat liver, and these findings suggest that oral administration of estrogens may induce hepatic dysfunctions and play a role in the development of liver disease.

Key Words: *Estriol; Metabolism; Kupffer cells; Oxygen consumption; Calcium*

Jin-Mo Yang, Sung-Soo Kim, Jin-Il Kim, Byung-Min Ahn, Sang-Wook Choi, Jae-Kwang Kim, Chang-Don Lee, Kyu-Won Chung, Hee-Sik Sun, Doo-Ho Park, Ronald G. Thurman*

Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea
Department of Pharmacology*, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.

Received: 3 November 1998
Accepted: 28 December 1998

Address for correspondence
Jin-Mo Yang, M.D.
Department of Internal Medicine, College of Medicine, The Catholic University of Korea, St. Paul's Hospital, 620-56, Jeonnong-dong, Dongdaemoon-gu, Seoul 130-707, Korea
Tel: +82.2.958-2114, Fax: +82.2-968-7250
E-mail: jmyang@sph.cuk.ac.kr

*This study was supported by research funds provided by St. Paul's Hospital.

INTRODUCTION

Various steroidal hormones are capable of inducing host changes and neoplasia. Estrogens are a group of 18-carbon steroids secreted primarily by the ovary and, to a lesser extent, the adrenals in females, and by the adrenals and testes in males. There is also a peripheral conversion of androgens to estrogens in adipose and muscle tissues (1). Differential sex characteristics among males and females and maintenance of reproductive organs are the result of differing ratios of estrogen to androgen. The major estrogen is estradiol, while other less potent estrogens include estrone and estriol. Estrogens may have the most diversified biological effects on tissue growth and the genesis of neoplasia (2). Moreover, the biological action of estrogen in the liver has remained relatively unclear. Kupffer cells and, to a lesser extent, sinusoidal endothelial cells play an important role in defending the host by phagocytizing foreign particles from portal blood.

Kupffer cells have long been known to play an important role in the phagocytosis of foreign particles. Carbon particles are taken up by Kupffer cells (3). Colloidal carbon is often used to monitor phagocytic cell function, since 90% is taken up by the liver after injection in vivo (3, 4). It has been demonstrated that during phagocytosis, Kupffer cells undergo an oxidative burst that leads to the production of reactive oxygen intermediates (5). In addition, nonparenchymal cells also secrete a wide variety of paracrine mediators, including cytokines, prostaglandins, and leukotrienes, which can modulate hepatocyte metabolism (6-9). Since estrogen is an important female sex hormone and leading to increased toxic mediator production by the liver (10), the present study was performed to assess the influence of exogenous estrogen in vivo on hepatic metabolism in the female rat liver.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley rats (175-200 g) received laboratory food and tap water ad libitum and housed with

a 12-hr day/night cycle. To study ketone body formation, rats were not fed for 24 hr before estriol treatment. After treatment, rats were not fed for another 24 hr before the experiments with perfused livers.

Materials

Rats were given an intraperitoneal injection of estriol (20 mg/kg body weight, Sigma, St. Louis, MO) 24 hr prior to experiments. All control rats received saline vehicle without estriol. $GdCl_3$ (10 mg/kg) dissolved in acidified saline (pH 3.0) was injected into the tail vein 24 hr prior to estriol treatment. Ethanol (5.0 g/kg) was administered intragastrically 2.5 hr prior to perfusion. All other chemicals were reagent grade from standard sources.

Liver perfusion

Well-fed female Sprague-Dawley rats (175-200 g) were anesthetized with pentobarbital sodium (50 mg/kg body wt). The abdomen was opened, and oxygen-saturated (95% O_2 : 5% CO_2) Krebs-Henseleit-bicarbonate buffer (37℃, pH 7.6) was pumped through the liver via a cannula inserted into the portal vein. A cannula placed in the inferior vena cava allowed fluid to flow out of the liver in a nonrecirculating manner. Oxygen concentration in the effluent perfusate was monitored with a Teflon-shielded, Clark-type oxygen electrode. Rates of oxygen uptake and metabolite production were caculated from influent minus effluent concentration differences, liver wet weight, and flow rate.

Metabolic assay

Glucose, glycolysis (lactate and pyruvate) and ketone bodies (acetoacetate and β-hydroxybutyrate) were assayed in the effluent perfusate by standard enzymatic techniques and rates of ketogenesis were calculated from concentrations of ketone bodies in the effluent perfusate, flow rate, and wet liver weight. Samples of effluent perfusate were collected every four min to determine metabolites. The first sample was collected at 12 min after initial perfusion. The ratios of lactate to pyruvate and β-hydroxybutyrate to acetoacetate were used to monitor the oxidation-reduction status of the extra- and intramitochondrial compartments, respectively (11).

Preparation of colloidal carbon

A suspension of colloidal carbon was prepared by dialyzing 25-30 mL of india ink against distilled water for 48 hr using a Spectrapor semipermeable membrane with a 12,000-14,000 molecular weight exclusion cut-off (Spectrum Medical industries, Los Angeles, CA). The suspension was stored at 4℃ for up to one month before use. In order to examine the phagocytic function of the reticuloendothelial system of liver, carbon was injected. On the day of the experiment, the carbon suspension was thoroughly stirred, sonicated and diluted with the same amounts of saline. Diluted carbon (2 mL/kg body wt) was injected into rat tail vein. Rats were sacrificed at 30 min after injection. The liver was taken out for histologic evaluation.

Kupffer cell isolation and culture

Kupffer cells from estriol-treated or control rats were isolated by collagenase digestion and put to differential centrifugation using Percoll (Pharmacia, Uppsala, Sweden) as described elsewhere with slight modifications (12). Briefly, the liver was perfused through the portal vein with Ca^{2+}-and Mg^{2+}-free Hanks' balanced salt solution (HBSS) containing 0.5 mM ethylene glycol-bis (β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) at 37℃ for five min at a flow rate of 24 mL/min. Subsequently, perfusion was done with HBSS containing 0.025% collagenase IV (Sigma Chemical Co., St. Louis, MO) at 37℃ for five min. After the liver was digested, it was excised and cut into small pieces in collagenase buffer. The suspension was filtered through a nylon gauge, and the filtrate was centrifuged twice at 50×g at 4℃ for three min to remove parenchymal cells. The non-parenchymal cell fraction was washed with buffer and centrifuged on a density cushion of Percoll at 1,000×g for 15 min to obtain the Kupffer cell fraction, followed by washing with buffer again. The viability and purity of isolated Kupffer cells were determined by trypan blue exclusion; values exceeded 90% and 85%, respectively. Cells were seeded onto 25 mm glass coverslips in 60 mm tissue culture dish (Corning Inc., Corning, NY) at a concentration of 5×10^5 and cultured in Dulbecco's modified Eagle's medium (D'MEM, GIBCO Laboratories Life Technologies Inc., Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 U/mL of penicillin G and 100 μg/mL of streptomycin sulfate) at 37℃ with 5% CO_2. Non-adherent cells were removed after one hr by replacing the culture medium. All adherent cells were phagocytosed latex beads, to indicate they were Kupffer cells. Cells were cultured for 24 hr prior to experiments.

Measurement of intracellular Ca^{2+}

Intracellular Ca^{2+} in individual Kupffer cells was measured fluorometrically using the fluorescent calcium indi-

cator dye fura-2 and a microspectrofluorometer (Photon Technology International, South Brunwick, NJ) interfaced with an inverted microscope (Diaphot, Nikon, Japan). Kupffer cells cultured on coverslips were incubated in modified Hanks' buffer (115 mM NaCl, 5 mM KCl, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 5.6 mM glucose, 0.8 mM $MgSO_4$, 1.26 mM $CaCl_2$, 15 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid, pH 7.4) containing 5 μM fura-2 / acetoxymetyl ester (Molecular probes Inc, Eugene, OR) and 0.03% Pluronic F127 (BASF Wyandotte, Wyandotte, MI) at room temperature for 60 min. Coverslips plated with Kupffer cells were rinsed and placed in chambers with buffer at room temperature. Changes in fluorescence intensity of fura-2 at excitation wavelengths of 340 nm and 380 nm and emission at 510 nm were monitored in individual Kupffer cells. Each value was corrected by subtracting the system dark noise and autofluorescence, assessed by quenching fura-2 fluorescence with Mn^{2+} as described previously (13). Intracellular Ca^{2+} was determined from the equation:

$$[Ca^{2+}]_I = Kd\{(R-R_{min})/(R_{max}-R)\}(F_o/F_s)$$

where F_o/F_s is the amount of fluorescent intensities evoked by 380 nm light from fura-2 pentapotassium salt loaded in cells using a buffer containing 3 mM EGTA and 1 μM ionomycin ($[Ca^{2+}]$ min) or 10 mM Ca^{2+} and 1 mM ionomycin ($[Ca^{2+}]$ max). R is the ratio of fluorescent intensities at excitation wavelengths of 340 nm and 380 nm, and Rmax and Rmin are values of R at $[Ca^{2+}]$ max and $[Ca^{2+}]$ min, respectively. The values of these constants were determined at the end of each experiment and a dissociation constant of 135 nM was used (14).

Statistics

Results are presented as mean±S.E.M. Analysis of repeated measurements was performed by a two-way RM ANOVA. Difference between the means of paired data with Student's paired t-tests, $*p<0.05$ was considered statistically significant.

RESULTS

Effect of estriol, ethanol, gadolinium chloride treatment on oxygen uptake

Basal rates of oxygen uptake in control, estriol-treated rats were around 114, 146 μmole/g/hr after 24 min of perfusion, respectively (Fig. 1). In ethanol-treated rats, the basal oxygen uptake was 152 μmole/g/hr. Combined-treatment with estriol and ethanol, oxygen uptake was

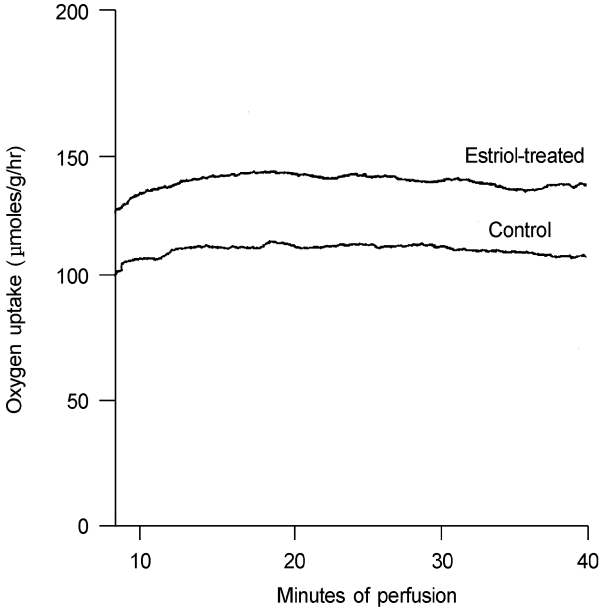

Fig. 1. Effect of estriol on rates of oxygen uptake in perfused livers. Livers from fed rats were perfused with Krebs-Henseleit bicarbonate buffer (pH 7.6, 37℃) in a noncirculating system as described in Methods. Estriol (20 mg/kg) was injected intraperitoneally 24 hr prior to perfusion. Rates were calculated from influent minus effluent concentration differences, flow rate and liver wet weight. Results are from typical experiments that were repeated eight times in each group.

158 μmole/g/hr. There were no additive effect between estriol and ethanol. However, oxygen uptake in estriol-treated rats after $GdCl_3$, which inactivates Kupffer cells, were similar to the control group. Its oxygen consumption was at 114 μmoles/g/hr. Values in $GdCl_3$-treated rats did not increase significantly. Thus, the increase in oxygen uptake observed in livers from estriol-treated rats was reduced significantly by about 75% as a result of $GdCl_3$ treatment (Fig. 2).

Carbohydrate metabolism

Glucose production

In the control group, the perfusate glucose concentration (ca. between 75.7±4.4 and 58.4±3.1 μmole/g/hr) was slowly decreased during the perfusion (Fig. 3A). In the estriol-treated group, glucose concentrations (ca. between 64.8±4.8 and 40.4±3.7 μmole/g/hr) recorded lower than that of the corresponding times in the controls (Fig. 3A). This effect was significant ($p<0.05$).

Pyruvate and lactate

Lactate plus pyruvate production is a good index to measure the rate of glycolysis, since pyruvate concentrations are below the Km of pyruvate dehydrogenase under these conditions (15). In the control group, the perfusate

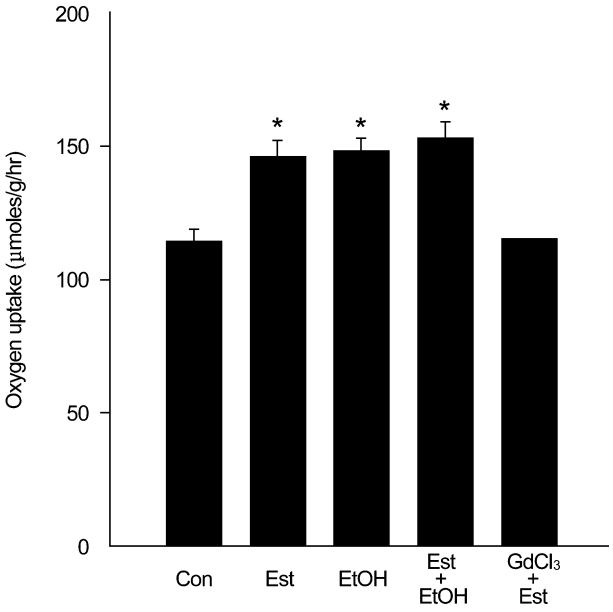

Fig. 2. Rates of basal oxygen uptake by perfused rat liver. Conditions as described in Fig. 1. Rats were treated with estriol (20 mg/kg i.p. 24 hr prior to perfusion) and ethanol (5.0 g/kg i.g. 2.5 hr prior to perfusion). In some experiments, $GdCl_3$ (10 mg/kg) was injected into the tail vein 24 hr prior to estriol treatment. Average rates of oxygen uptake were calculated at 24 min of liver perfusion from eight livers per treatment group. Rates were calculated from influent minus effluent concentration differences, flow rate and liver wet weight. Mean values are shown and bars indicate the S.E.M. Statistical comparisons used Student's t-test. *$p<0.05$ for comparison with appropriate control.

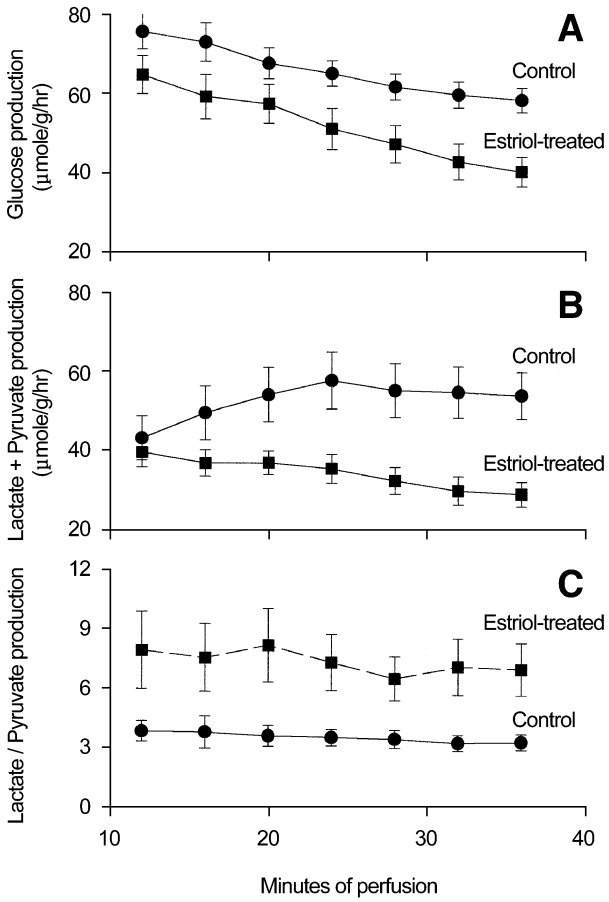

Fig. 3. Effect of estriol on carbohydrate production by perfused rat liver. A: Concentrations of glucose production after 12 min of liver perfusion. B: Concentrations of lactate plus pyruvate production after 12 min of liver perfusion. C: Ratio of perusate [lactate] / [pyruvate] after 12 min of liver perfusion. Rates were calculated from influent minus effluent concentration differences, flow rate and liver wet weight and expressed as μmole/g/hr. Results are from typical experiments that were repeated six times in each group. Mean values are shown and bars indicate the S.E.M. (n=6). Statistical significance for comparison with control rats by two-way RM ANOVA.

pyruvate plus lactate concentration (ca. between 43.1±5.6 and 53.9±5.9 μmole/g/hr) was increased slightly during the perfusion (Fig. 3B). However, in the estriol-treated group, the concentration (ca. between 39.5±3.8 and 29.0±3.1 μmole/g/hr) was slightly decreased. In all the groups of liver perfusions derived from estriol-treated rats, concentrations were lower than that of corresponding times in the controls. This effect was significant ($p<0.05$). The perfusate [lactate]/[pyruvate] ratios in estriol-treated group were between 6.4 and 8.1. In the control group, the ratio showed between 3.2 and 3.8 (Fig. 3C). The perfusate [lactate]/[pyru-vate] ratios in estriol-treated group were higher than the control groups ($p<0.05$).

Lipid metabolism

Acetoacetate and β-hydroxybutyrate

In the control group, the perfusate β-hydroxybutyrate plus acetoacetate concentrations (ca. between 21.82±1.65 and 17.65±2.24 μmole/g/hr) was slowly decreased during the perfusion (Fig. 4A). In the estriol-treated group, the concentrations (ca. between 32.38±1.13 and 29.24±1.06 μmole/g/hr) were higher than that of corresponding times in the controls (Fig. 4A). In perfusions of livers from estriol-treated rats, the β-hydroxybutyrate plus acetoacetate concentrations were significantly greater than in controls ($p<0.05$).

The [β-hydroxybutyrate]/[acetoacetate] ratios in the perfusate were between 0.98 and 0.24 in control perfusions (Fig. 4B). In the estriol-treated group, the ratios were between 0.4 and 0.24. After an equilibration period of initial 20 min perfusion, in the perfusions of livers from estriol-treated rats, the [β-hydroxybutyrate]/[acetoacetate] ratio was slightly higher than in controls (Fig. 4B).

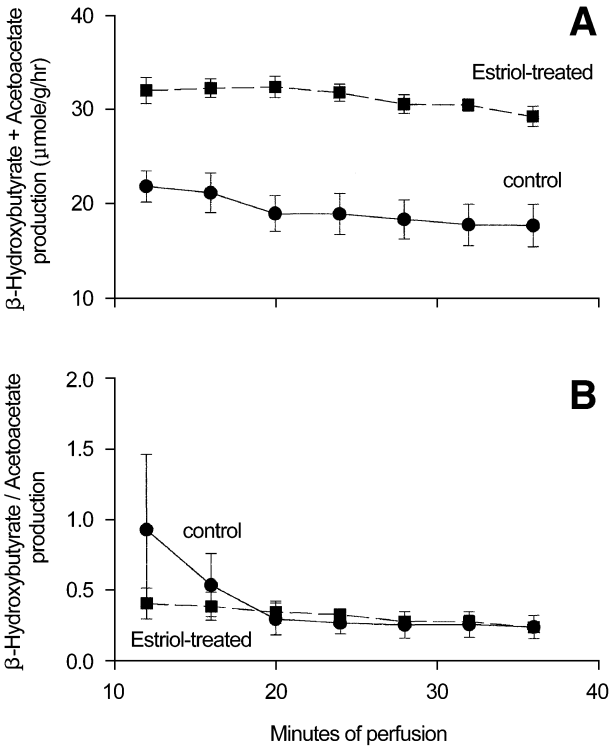

Fig. 4. Effect of estriol on ketone body production by perfused rat liver. A: Concentrations of β-hydroxybutyrate plus acetoacetate production after 12 min of liver perfusion. B: Ratio of perfusate [β-hydroxybutyrate] / [acetoacetate] after 12 min of liver perfusion. Rates were calculated from influent minus effluent concentration differences, flow rate and liver wet weight and expressed as μmole/g/hr. Results are from typical experiments that were repeated six times in each group. Mean values are shown and bars indicate the S.E.M. (n=6). Statistical significance for comparison with control rats by two-way RM ANOVA.

Phagocytosis of colloidal carbon

Histological sections of livers exhibited no detectable carbon in parenchymal cells. The difference in the development of sinusoids between the two groups was more evident when the rats received an intravenous injection of carbon. In the estriol-treated group, well-developed sinusoids were clearly lined by endothelial cells with carbon particles (Fig. 5).

To assess phagocytotic activity of Kupffer cells and reticuloendothelial cells, carbon uptake was measured in livers by computer image analysis. The intensity of carbon staining rose with increasing rates of carbon uptake in non-parenchymal cells scored histologically. Carbon uptake of the control group was showed to 0.95% (Fig. 6). Carbon uptake of estriol-treated group was marked to 3.15 (Fig. 6). Carbon uptake in livers increased in estriol-treated rats compared with control rats ($p<0.05$). Earlier studies have demonstrated that $GdCl_3$ inhibits phagocytosis by Kupffer cells in vivo (16). Carbon uptake of $GdCl_3$-pretreatment and estriol-treated group were 1.41 (Fig. 6). As expected, carbon uptake in $GdCl_3$-pretreated rats was lower than in estriol-treated rats (Fig. 6, $p<0.05$).

Intracellular Ca^{2+} in Kupffer cells

Intracellular calcium in individual cultured Kupffer cell was measured fluorometrically. As reported, LPS increased intracellular Ca^{2+} transiently in isolated Kupffer cells. After exposure to LPS, intracellular Ca^{2+} showed a rapid increase followed by a rapid decline. Intracellular Ca^{2+} with 1,000 ng/mL of LPS in isolated Kupffer cell from control, estriol-treated rats were showed to be around 195, 372 nM, respectively (Fig. 7). LPS increased intracellular Ca^{2+} in a dose-dependent manner.

The increase in intracellular Ca^{2+} due to LPS was significantly higher in Kupffer cells isolated from estriol-treated rats (Fig. 8).

DISCUSSION

Estrogens are well-known strong stimulants of phagocytic (17) and proliferative (18, 19) activities of the reticuloendothelial system (RES). Oral administration of natural estrogens caused not only the proliferation of Kupffer cells, but also hepatocyte proliferation without any obvious accompanying liver damage, such as degeneration or necrosis. The mechanism involved in the powerful stimulating effect of natural estrogens on the proliferation of hepatocytes is uncertain. Since hepatocytes have estrogen receptors (20), estrogens may directly affect hepatocytes to cause mitosis. Another possible interpretation of the mechanism of estrogen action is the close correlation between the proliferation of hepatocytes and activation of RES. Again, estrogens are strong stimulants of RES (17).

As for the effects of estrogens on hepatic endothelial cells, direct action by estrogens upon the blood vessels of hormone-dependent tumors should be considered in addition to their effect on tumor cells themselves (21).

Furthermore, it is well known that activated Kupffer cells release a number of mediators, such as prostaglandins (22), proteases, leukotrienes, tumor necrosis factor (23), and interleukins (24). Some of these mediators participate in mechanisms of intercellular communication in the liver. It is hypothesized that some mediators released from activated Kupffer cells regulate oxygen uptake in hepatocytes during estriol or ethanol treatment. To support this theory, we observed that the increase in oxygen uptake during estriol treatment was reduced by approxi-

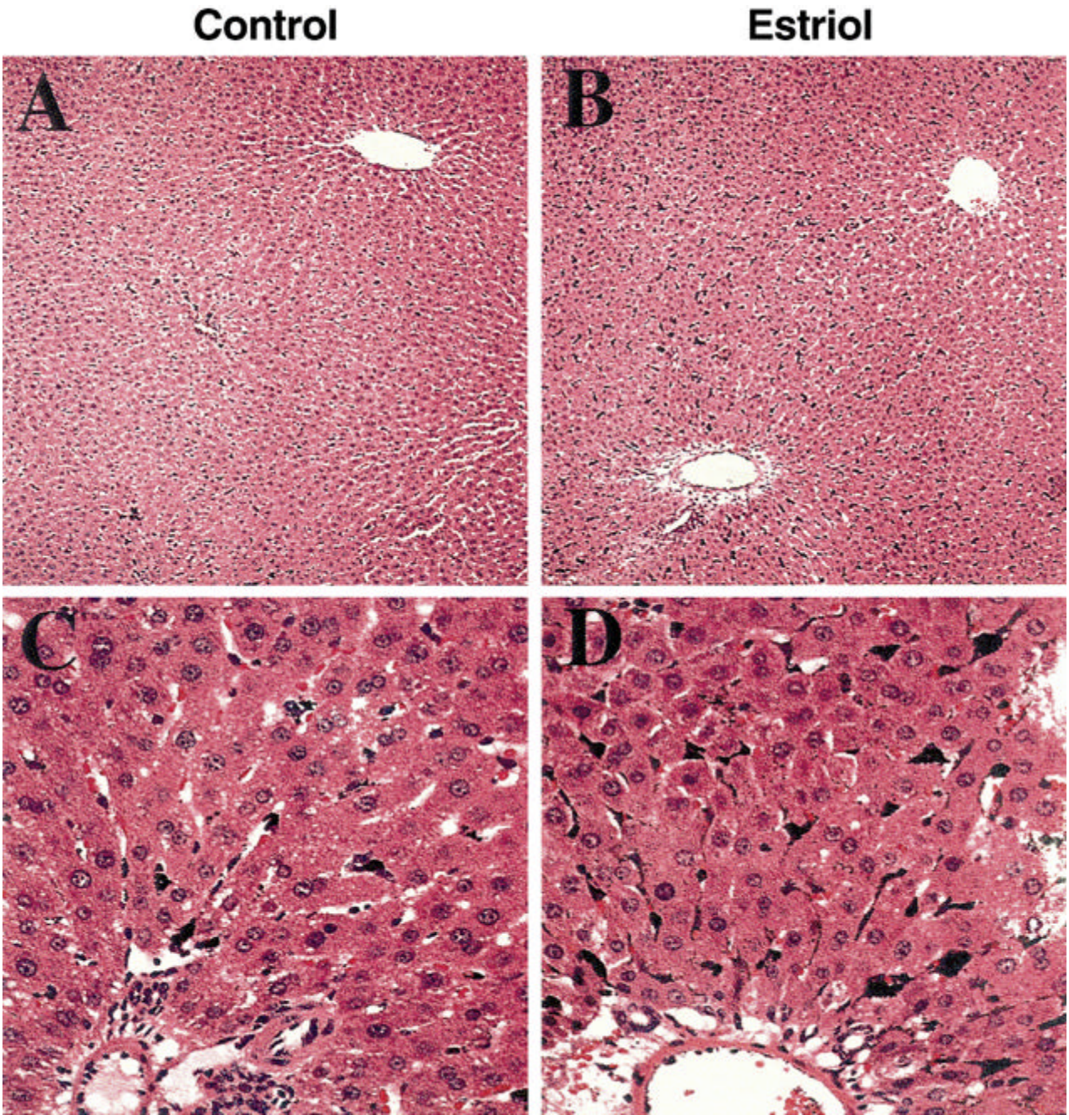

Fig. 5. Light micrographs of rat liver after intravenous injection of carbon. A: The thickest part of the liver in a control rat. Carbon-laden cells are scarce (H&E, ×100). B: The thickest part of the liver in a estriol-treated rat. Many Kupffer cells and endothelial cells stained black due to abundant phagocytized carbon can be seen in sinusoids (H&E, ×100). C: The thickest part of the liver in a control rat (H&E, ×400). D: The thickest part of the liver in a estriol-treated rat. Well-developed sinusoids are lined by carbon-laden endothelial cells. Many Kupffer cells stained black due to abundant phagocytized carbon can be seen in sinusoids (H&E, ×400).

mately 75% when Kupffer cells were inactivated by $GdCl_3$ treatment (Fig. 2).

Our results clearly show that estriol inhibits gluconeogenesis by shifting the NAD^+/NADH ratio towards reduction (as shown in Fig. 3). A shift in the mitochondrial oxidation-reduction state towards reduction is known to lower the rate of phosphoenolpyruvate formation by liver mitochondria and to be associated with a decreased rate of gluconeogenesis (25). Haeckel and Haeckel (26) demonstrated that biguanide can markedly shift the mitochondrial oxidation-reduction state of guinea pig liver towards reduction. Matute and Kalkhoff (27)

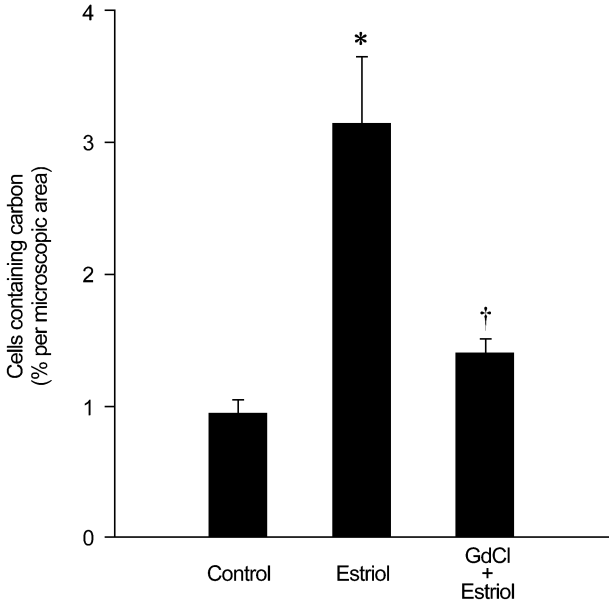

Fig. 6. Rates of carbon containing area after intravenous injection of carbon. The accumulation of carbon in liver tissue was quantitated using image analysis. Results are from typical experiments that were repeated four times in each group. Mean values are shown and bars indicate the S.E.M. (n=4). *$p<0.05$ compared to controls, and †$p<0.05$ compared to liver given estriol using one-way RM ANOVA with Kruskal-Wallis's multiple comparison test.

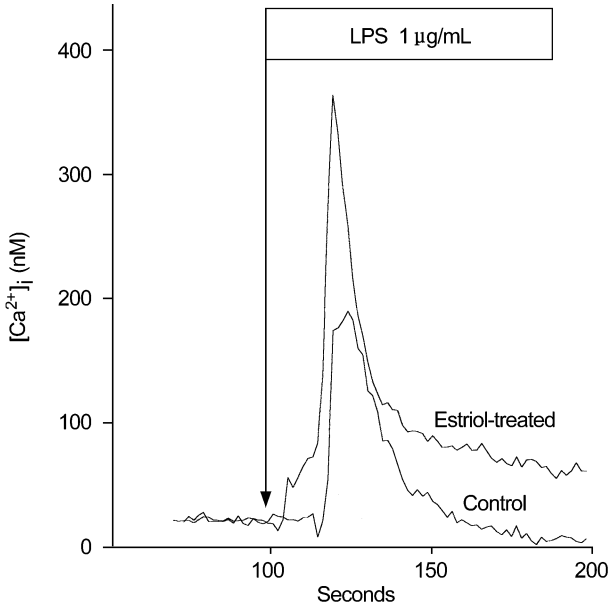

Fig. 7. Effect of estriol on LPS-induced increases on intracellular [Ca^{2+}] in cultured Kupffer cells. Intracellular calcium in cultured Kupffer cells was measured fluorometrically using the fluorescence indicator fura-2 as described in Methods. LPS (1 μg/mL) was added with 5% rat serum in modified HBSS. The data are representative of experiments repeated four times.

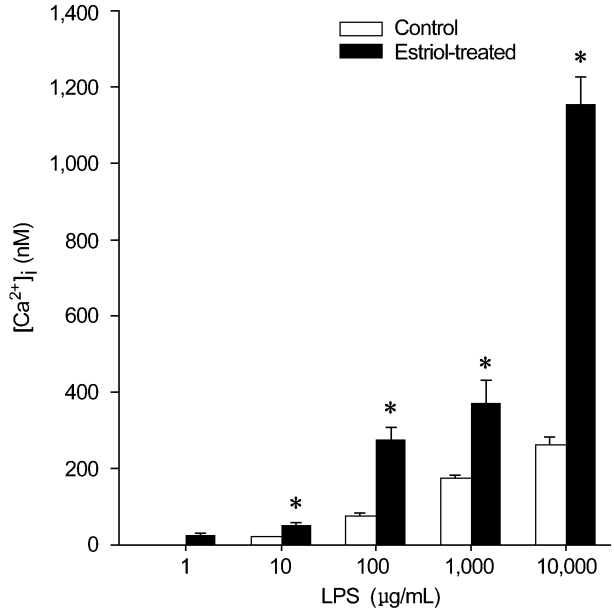

Fig. 8. Effect of estriol on dose-response of LPS on intracellular [Ca^{2+}] in cultured Kupffer cells. Conditions as described in Fig. 7, except that various doses of LPS (10-10,000 ng/mL) were used. Calcium levels were quantified by subtracting the basal level (20±5 nM) from peak values. Mean values are shown and bars indicate the S.E.M. (n=4). Statistical significance for comparison with control rats using one-way RM ANOVA with Kruskal-Wallis's multiple comparison test.

showed that estrogen suppresses hepatic gluconeogenesis while promoting liver glycogen deposition. This insulin-like effect can be related to induced hyperinsulinemia, suggesting that the liver does not recognize this sex steroid as insulin antagonists. The present study showed that hepatic glucose production in estriol-treated rats was decreased as compared with control rats.

Pyruvate is the immediate precursor of acetyl-CoA during the conversion of carbohydrate into fatty acids. The major fates of the acetyl group of acetyl-CoA are conversion into long chain fatty acids, β-hydroxybutyrate, acetoacetate and CO_2. In addition to measuring the rate of fatty acid synthesis, we also measured the rates of formation of pyruvate and lactate, and of β-hydroxybutyrate and acetoacetate as a function of the glucose concentration in the perfusate.

The decrease in lactate and pyruvate output in estriol-treated rats seen in our experiment indicates that the NAD^+/NADH ratio is displaced to a more negative value. The marked increase in the Lactate/Pyruvate ratio in estriol-treated rats can be explained on the basis of displacement of the NAD^+/NADH ratio in the cytosolic compartment. A significant increase in the Lactate/Pyruvate ratio was indicative of a less oxidized redox state in the cytosol. Subsequently, the rate of glycolysis at the glyceroaldehyde 3-phosphate dehydrogenase step was

decreased. A decrease in NADPH concentration in the liver of estrogen-treated rats may also limit the conversion of hydroxymethylglutaryl-CoA by NADPH-dependent microsomal reductase (28).

The increased glycogenolytic response of the liver to prostaglandins has been shown to be due to the increased mobilization of Ca^{2+} stores (29). Mobilized Ca^{2+} activates pyruvate, isocitrate and 2-oxoglutarate dehydrogenases (30). This leads to an increased production of NADH and an increase in O_2 consumption by hepatocytes. Estriol-treated group showed to significantly increase in the rate of O_2 uptake by the perfused liver.

In hepatocytes, the lactate-pyruvate system provides the most reliable value for cytosolic NAD^+/NADH while the β-hydroxybutyrate:acetoacetate ratio is a measure of the mitochondrial redox state (31). In normal cells, coupling of cytosolic and mitochondrial processes ensures that NADH does not accumulate and that the difference in redox potential between the two cellular compartments is maintained. The increase in ketogenesis in liver perfusions of estriol-treated rats was due mainly to augmented production of acetoacetate rather than β-hydroxybutyrate. This may be related to the increased capacity of peroxisomes to oxidize fatty acids. Acetoacetate may be formed directly by peroxisomes, whose oxidation pathway would favour acetoacetate production, since β-hydroxybutyrate dehydrogenase, which is required to convert acetoacetate into β-hydroxybutyrate, is located exclusively in the mitochondria (32).

The important problem of whether estrogen acts on hepatocytes directly or not is also unresolved. Since hepatocytes have estrogen receptors (20, 33) it is thought that the proliferative effect of estriol on the hepatocytes may be a direct action. However, the sinusoidal vascular supply is essential to the proliferation of hepatocytes. The effect of estrogens on the proliferation of sinusoidal endothelial cells and Kupffer cells in the adult liver has been previously reported (21, 34). As for the effects of estrogens on hepatic endothelial cells, the higher frequency of endothelial mitosis in central rather than in periportal zones of the liver lobule is most probably related to the difference in distribution and ratio of Kupffer cells to endothelial cells in different parts of the hepatic lobule. The inhibition of Kupffer cell phagocytosis by gadolinium chloride may be related to the replacement of calcium at the surface membrane site of the Kupffer cells. In this study, the development of sinusoids in the liver tissue was much more progressive in the estriol-treated group than in the control group.

Since changes in intracellular Ca^{2+} are most likely important in the production of chemical mediators in Kupffer cells, the effect of estrogen on intracellular Ca^{2+} due to LPS was assessed in isolated Kupffer cells (Fig. 7, 8). The intracellular Ca^{2+} due to LPS was significantly higher in Kupffer cells isolated from estriol-treated rats. The mechanism of estriol effect in Kupffer cells is unknown; however, estriol treatment induced sensitization of Kupffer cells.

In conclusion, these findings suggest that the metabolic effects of estriol (2 mg per 100 mg body wt) can be summarized to be highly toxic in rat liver, and these findings suggest that oral administration of estrogens may induce hepatic dysfunctions and play a role in the development of liver disease.

REFERENCES

1. Luocks AB, Harvath SM. *Athletic amenorrhea: a review. Med Sci Sports Exerc 1985; 17: 56-72.*
2. Dao TL. *Metabolism of estrogens in breast cancer. Biochem Biophys Acta 1979; 560: 397-426.*
3. Triarhou LC, del Cerro M. *Colloidal carbon as a multi-level marker for experimental lesions. Experientia 1985; 41: 620-1.*
4. Matsuo S, Nakagawara A, Ikeda K, Mitsuyama M, Nomoto K. *Enhanced release of reactive oxygen intermediates by immunologically activated rat Kupffer cells. Clin Exp Immunol 1985; 59: 203-9.*
5. Bhatnagar F, Schirmer R, Ernest M, Decker K. *Superoxide release by zymosan-stimulated rat Kupffer cells in vitro. J Biochem 1981; 119: 171-5.*
6. Kuiper J, Casteleyn E, Van Berkel JC. *Regulation of liver metabolism by intercellular communication. Adv Enzyme Regul 1988; 27: 193-208.*
7. Altin JG, Bygrave FL. *Non-parenchymal cells as mediators of physiological responses in the liver. Mol Cell Biochem 1988; 83: 3-14.*
8. Nathan CF. *Secretory products of macrophages. J Clin Invest 1989; 79: 319-26.*
9. Decker K. *Biologically active products of stimulated liver macrophages (Kupffer cells). Eur J Biochem 1990; 192: 245-61.*
10. Ikejima K, Enomoto N, Imuro Y, Ikejima A, Fang D, Xu J, Forman DT, Brenner DA, Thurman RG. *Estrogen increases sensitivity of hepatic Kupffer cells to endotoxin. Am J Physiol 1998; 274: G669-76.*
11. Veech RL, Raijman L, Krebs HA. *Equilibrium relations between the cytoplasmic adenine nucleotide system and nicotinamide adenine nucleotide system in rat liver. Biochem J 1970; 117: 499-503.*
12. Grynkiewicz G, Poenie M, Tsien RY. *A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J Biol Chem 1985; 260: 3440-50.*
13. Hijioka T, Rosenberg RL, Lemasters JJ, Thurman RG. *Kupffer cells contain voltage-dependent calcium channels. Mol Pharmacol 1992; 41: 435-40.*

14. Nolan JP, Ali MV. *Late effect of estrogen on endotoxin response in the rat. J Lab Clin Med 1968; 71: 501-10.*
15. Thurman RG, Scholz R. *Interaction of glycolysis and respiration in perfused rat liver. Changes in oxygen uptake following the addition of ethanol. Eur J Biochem 1977; 75: 13-21.*
16. Bradford BU, Marotto ME, Lemasters JJ, Thurman RG. *New, simple models to evaluate zone-specific damage due to hypoxia in the perfused rat liver: time course as effect of nutritional state. J Pharmacol Exp Ther 1986; 236: 263-8.*
17. Vernon-Roberts B. *The effects of steroid hormones on macrophage activity. Int Rev Cytol 1969; 25: 131-59.*
18. Kelly LS, Dobson EL. *Evidence concerning the origin of liver macrophages. Br J Exp Path 1971; 52: 88-99.*
19. Sljivic VS, Warr GW. *Role of cellular proliferation in the stimulation of MPS phagocytic activity. Br J Exp Path 1975; 56: 314-21.*
20. Eisenfeld AJ, Weinberger RAM, Haselbacher GK, Halpern K. *Estrogen receptor in the mammalian liver. Science 1976; 191: 862-5.*
21. Widmann JJ, Fahimi HD. *Proliferation of endothelial cells in estrogen-stimulated rat liver. Lab Invest 1976; 34: 141-9.*
22. Tran-Thi TA, Gyufko K, Haussinger D, Decker K. *Net prostaglandin release by perfused rat liver after stimulation with phorbol 12-myristate 13-acetate. J Hepatol 1988; 6: 151-7.*
23. Bouwens L. *Structural and functional aspects of Kupffer cells. Rev Biol Cell 1988; 16: 69-94.*
24. Chensue SW, Terebuh PD, Remick DG, Scales WE, Kunkel SL. *In vivo biologic and immunohistochemical analysis of interleukin-1 alpha, beta and tumor necrosis factor during experimental endotoxemia. Kinetics, Kupffer cell expression, and glucocorticoid effects. Am J Pathol 1991; 138: 395-402.*
25. Garber AJ, Hanson RW. *The control of phosphoenolpyruvate formation of rabbit liver mitochondria. J Biol Chem 1971; 246: 5555-62.*
26. Haeckel R, Haeckel H. *Inhibition of gluconeogenesis from lactate by phenylethylbiguanide in the perfused guinea pig liver. Diabetologia 1972; 8: 117-24.*
27. Matute ML, Kalkhoff RK. *Sex steroid influence on hepatic gluconeogenesis and glycogen formation. Endocrinology 1973; 92: 762-8.*
28. Mukherjee R, Bhose A. *Studies on estrogen regulation of cholesterol biosynthesis in rat liver microsomes. Biochim Biophys Acta 1968; 164: 357-68.*
29. Altin JG, Bygrave FL. *Prostaglandin F2a and the thromboxane A2 analogue ONO-11113 stimulate Ca^{2+} fluxes and other physiological responses in rat liver. Biochem J 1988; 249: 677-85.*
30. McCormack JG. *Characterization of the effects of Ca^{2+} on the intramitochondrial Ca^{2+}-sensitive enzymes from rat liver and within intact rat liver mitochondria. Biochem J 1985; 231: 581-95.*
31. Williamson DH, Lund P, Krebs HA. *The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem J 1967; 103: 514-27.*
32. Krebs HA. *In rate control of biological processes. Cambridge University Press, 1974: 299-324.*
33. Francavilla A, di Leo A, Eagon PK, Wu SQ, Ove P, van Thiel DH, Starzl TH. *Regenerating rat liver: correlations between estrogen receptor localization and deoxyribonucleic acid synthesis. Gastroenterology 1984; 86: 552-9.*
34. Fujii H, Hayama T, Kotani M. *Stimulating effect of natural estrogens on proliferation of hepatocytes in adult mice. Acta Anat 1985; 121: 174-8.*