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FACTORS AFFECTING HYPERTRIGLYCERIDEMIA DURING PREGNANCY

By

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ABSTRACT

FACTORS AFFECTING HYPERTRIGLYCERIDEMIA
DURING PREGNANCY

By

Helen Jean Palmer

Rats were ovariectomized and injected daily for 21 days with sesame oil (control, C) or 1 ug estradiol (E) and/or 2 mg progesterone (P). Plasma triglycerides (TG) were greater ($P < 0.05$) in E or E+P treated rats than C. Parametrial adipose lipoprotein lipase activity (LPLA) was lower in the E and E+P groups than C on a total tissue basis. When LPLA was expressed on a DNA basis, no differences were found.

In experiment 2, ovariectomized rats injected daily with E+P were injected with saline or 0.2 mg 2-bromo-α-ergocryptine (CB-154) and/or 50 I.U. prolactin. CB-154 is an inhibitor of prolactin secretion. No differences were found in plasma TG, adipose LPLA or liver TG synthesis rate after a 12-hour fast.

In experiment 3, pregnant rats were injected with saline (I), CB-154 (II) or CB-154 and prolactin (III). Food intake was restricted after day 12 to the amount consumed by 12-day pregnant rats. Fasting plasma TG on days

19 and 21 were 300% ($P < 0.05$) of days 0 and 12 while adipose LPLA per unit DNA on days 19 and 21 were 30% ($P < 0.05$) of days 0 and 12. Liver TG synthesis rates were not different between any of the times. Placental LPLA was greater on days 19 and 21 than on day 12. Groups I and III had similar plasma TG which were greater than those of group II on days 19 and 21. No treatment differences in the pregnant rats were found for any tissue LPLA or for liver TG synthesis. The results indicate hypertriglyceridemia occurs during pregnancy even when energy is restricted to the amount consumed by 12-day pregnant rats and that E promotes increased plasma TG by decreasing adipose LPLA. Inhibition of prolactin secretion causes an increase in plasma TG which returns to the control level when exogenous prolactin is injected with CB-154. The change in plasma triglycerides caused by prolactin did not correlate with tissue LPLA or liver TG synthesis.

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INTRODUCTION

Mortality and morbidity due to premature coronary heart disease is a major health problem in America. Hypertriglyceridemia associated with hypercholesterolemia has been indicated as a risk factor in coronary heart disease (1). Premenopausal women have a lower risk of coronary heart disease than post-menopausal women. It is believed that physiological levels of estrogen have a protective effect against coronary heart disease, however high levels of estrogen promote an increase in the blood triglyceride concentration. Women taking oral contraceptive pills have increased blood triglyceride concentrations and have an increased risk of myocardial infarctions (2). The current concern with preventing coronary heart disease has lead to much discussion on what changes need to be made in the American lifestyle to lower the risk of coronary heart disease. Dietary manipulation, physical activity and pharmaceutical intervention have received the main emphasis in lowering blood cholesterol and triglycerides and presumably coronary heart disease. For women using oral contraceptives, the influence of hormonal preparations on triglyceride metabolism should be considered also.

Hypertriglyceridemia during the last half of pregnancy appears to be a normal physiological response. However, the mechanism(s) involved in producing hypertriglyceridemia is (are) not well understood. Hormonal changes and/or an increase in food consumption may be causing changes in lipid metabolism. The research for this thesis was conducted to investigate the extent of the contribution of hormonal influence and diet to hypertriglyceridemia during pregnancy. The knowledge might also be applied to reducing the hypertriglyceridemia risk factor of coronary heart disease.

LITERATURE REVIEW

Hypertriglyceridemia has been observed during the last half of pregnancy in the human (3), rat (4,5,6,7), and dog (8). Increased serum triglycerides are seen from the fifth month of pregnancy until parturition in humans. In the rat, increased triglyceride concentrations begin on day 12 and continue through day 20 just prior to parturition on day 21 or 22.

Increased blood triglycerides could result from decreased triglyceride removal from the blood and/or increased triglyceride mobilization into the blood. Removal of triglycerides from the blood occurs when tissue lipoprotein lipase (LPL) hydrolyzes the triglycerides carried in chylomicrons and very low density lipoproteins (VLDL). The fatty acids are then taken up by the tissue and used for energy or stored as triglycerides. Tissue LPL is associsted with endothelial cells of capillary walls in the heart, lung, mammary gland, spleen, muscle, and adipose tissue. Liver contains hepatic lipase, which is distinct from other tissue LPL.

An intravenous injection of heparin releases LPL and hepatic lipase into the blood. Post-heparin lipase

activity (PHLA) has been used as a measurement of lipase activity. The amount of enzyme released is proportional to the heparin dosage, however it is not clear if the releasable pool of enzyme reflects in vivo activity.

A technical problem with measuring PHLA is the difference in enzyme specificity for the substrate used in the assay. Knopp et al. (7) measured PHLA after sham operating or partially hepatectomizing rats 35-38% or 59-69%. By extrapolating to 100% hepatectomy, they found liver PHLA was greater when Intralipid, a soybean oil-egg lecithin emulsion similar to chylomicrons, was used as the substrate than when Ediol, a coconut oil emulsion was the substrate. Unless an inhibitor is used in the assay for hepatic lipase or LPL, it is not possible to attribute a decrease in PHLA to either enzyme. This problem is important to consider when interpreting data in the literature.

Increased mobilization of triglycerides into the blood, which could produce hypertriglyceridemia, may occur when substrate availability for triglyceride synthesis is high and the activity of enzymes involved in triglyceride synthesis increase. The increase in food consumption during the last half of pregnancy could provide more substrate for triglyceride synthesis.

Triglyceride synthesis occurs in the liver when energy intake is high or when fasting has increased the influx of fatty acids mobilized from the adipose tissue.

Carbohydrate is converted to acetyl CoA which is used to synthesize fatty acids. These fatty acids and fatty acids from hydrolyzed triglycerides are esterfied to glycerol-3-phosphate in a series of steps to produce triglycerides (Figure 1). The triglycerides combine with phospholipids, cholesterol and apoproteins synthesized in the liver to form VLDL which are secreted into the blood stream.

Hypertriglyceridemia During Pregnancy

PHLA has been used to measure triglyceride removal rates during pregnancy. Fabian et al. (9) reported women pregnant 33-38 weeks had lower PHLA than non-pregnant women. No distinction was made between hepatic lipase and LPL in the assay system since Infonutrol (a fat emulsion containing 15% cottonseed oil) was used as a substrate. There was no distinct enzyme specificity for this substrate. The increased blood volume which normally occurs during pregnancy was not considered either. Knopp et al. (7) found lower PHLA, which was corrected for the increase in plasma volume, in the fed rat on day 21 of pregnancy compared to day 12 or 19 when the substrate was Ediol. When Intralipid was used as the substrate, a significant increase in PHLA was found on day 12. By day 19, the activity had declined and was significantly less than the PHLA of 12-day pregnant rats but not non-pregnant rats. PHLA of 21-day pregnant rats

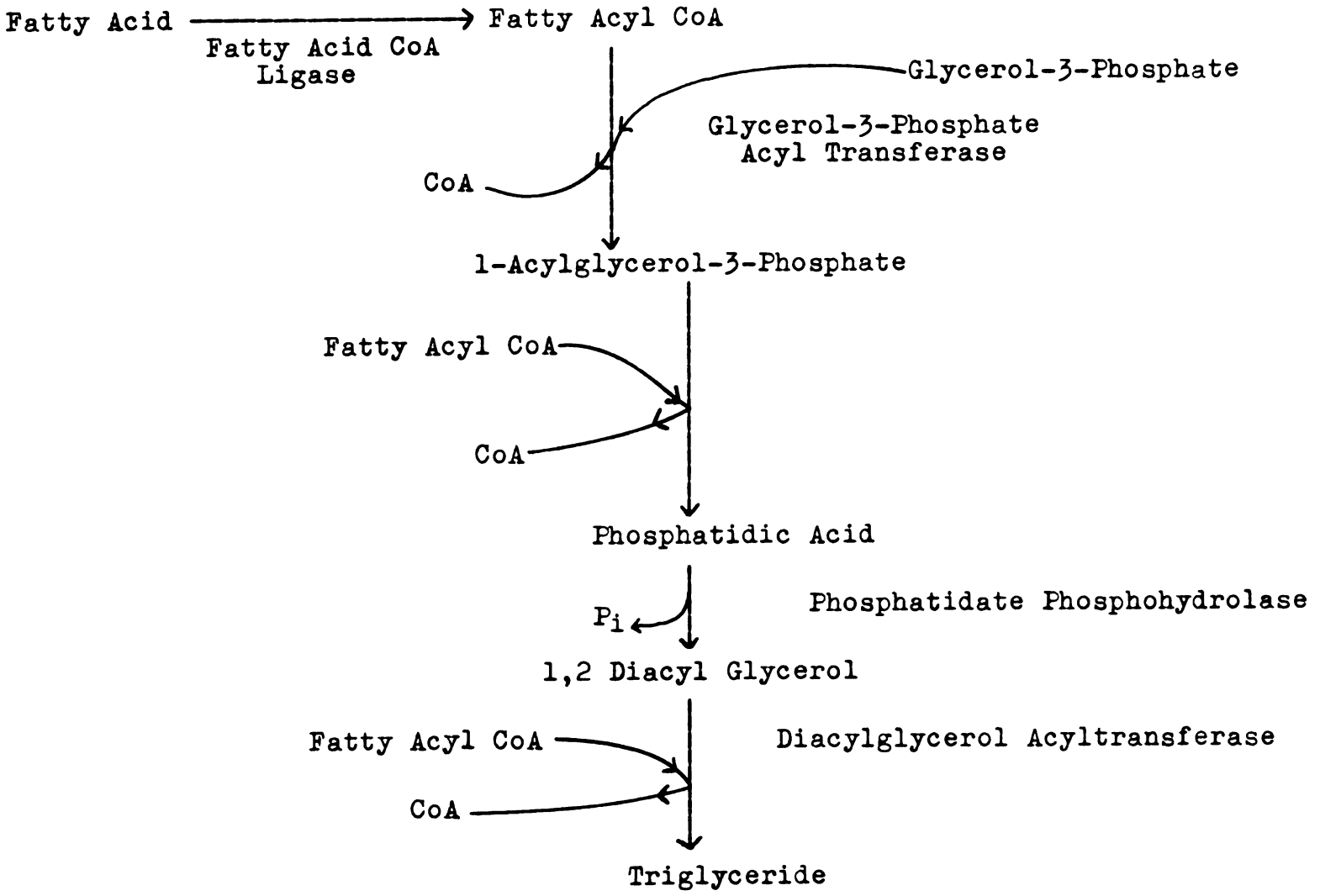

Fig. 1. Synthesis of Triglycerides from Fatty Acids and Glycerol-3-Phosphate.

was significantly lower than 19-day pregnant and non-pregnant rats. From the differences found in enzyme specificity for the two substrates, the hepatic lipase appears to increase during the first half of pregnancy while LPL remains constant. During the second half of pregnancy both enzyme activities decrease. The adipose tissue LPLA of 19- and 21-day pregnant rats did parallel the decrease in PHLA.

Otway and Robinson (5) and Hamosh et al. (6) measured adipose tissue LPLA of rats during pregnancy and found significantly less activity on days 20 or 21 of pregnancy compared to virgin rats. Both investigators reported a steady decrease in LPLA from day 13 to 19, but the activity was never significantly different from virgin rats. Hamosh et al. (6) did find a significant increase in the activity on day 12, which agrees with the results of the PHLA reported by Knopp et al. (7) when Intralipid was used as the substrate. Both Otway and Robinson (5) and Hamosh et al. (6) reported an increase in the serum triglyceride level occurring with the decrease in LPLA. However, the serum triglyceride level rapidly declined after day 20 even though LPLA was very low. At this time food intake was reported to decrease significantly. When food intake declines and substrate for triglyceride synthesis is decreased, serum triglyceride concentration would be expected to decrease. The increase in mammary gland LPLA at this time

reported by Hamosh et al. (6) may also contribute to the decrease in serum triglyceride concentration.

There are few reports on triglyceride production during pregnancy. Dannenburg et al. (10) found increased incorporation of ^{14}C-acetate into fatty acids of fed pregnant rat liver slices compared to incorporation of non-pregnant rat liver slices. However, the calculations were based on the specific activity of exogenous acetate and did not account for any differences between endogenous pool sizes which were probably present in the fed state. Otway and Robinson (5) measured the rate of triglyceride influx after a Triton WR1339 injection, which inhibits LPL and the removal of triglycerides from the blood. The rate of triglyceride entry into the plasma gradually increased from days 10-15 to days 21-22 when expressed per rat. When the rate was expressed per 200 grams of body weight, the size of a non-pregnant rat, no differences were found. The increase in triglyceride mobilization reported indicates that hypertriglyceridemia occurs as a result of the increased body size during pregnancy.

A relationship between dietary intake and hypertriglyceridemia during pregnancy has been reported. Scow et al. (4) reported that increased food consumption paralleled increased triglyceride concentrations after day 12. The effect of fat content in the diet was also investigated. When rats were switched to a "fat-free" diet (0.15% methyl

linoleate) on day 16 the blood triglyceride concentration decreased 75 mg % by day 20, while rats on the stock diet (4.5% fat) had a 125 mg % increase. In contrast, Otway and Robinson (5) reported similar increases in serum triglycerides in rats fed a "fat-free" diet (0.3% corn oil) as those fed a stock diet (3% digestible fat) after day 12. In both of these studies, other components of the diet were varied and could have affected lipid metabolism.

From the reports on triglyceride removal from the blood and liver triglyceride synthesis during pregnancy, hypertriglyceridemia may be the result of increased synthesis when food consumption is increased. Decreased LPLA appears to contribute to the hypertriglyceridemia during the latter part of pregnancy.

Influence of Sex Steroid Hormones on Blood Triglyceride Concentration

During pregnancy, the hormonal balance is changing. The growing placenta produces estrogen, progesterone and placental lactogen in increasing amounts as pregnancy progresses both in humans (11,12,13) and rats (14,15). The change in blood concentration of these hormones corresponds with the hypertriglyceridemia. Several investigators have looked at the influence of estrogen and progesterone on the mechanism of hypertriglyceridemia.

The effect of estrogen and progesterone in the contraceptive pill on PHLA has been studied. Ham and Rose (16) reported decreased PHLA in women taking the pill containing both estrogen and progesterone for at least 6 months. Hazzard et al. (17) measured PHLA in women taking ethinyl estradiol and medroxyprogesterone for 2 weeks. A significant decrease in PHLA occurred while plasma triglycerides were significantly increased. Plasma insulin was also increased in the women taking the oral contraceptive. Administration of exogenous insulin, which stimulates adipose LPLA, did not increase the PHLA. From the decreased PHLA, they concluded the increase in triglycerides may be caused by a decrease in the removal or the hyperinsulinemia may promote an increase in synthesis of triglycerides or both.

PHLA may not be a valid way to assess LPLA and the relationship to triglyceride removal from the blood. Hazzard et al. (18) found no differences in oral fat tolerance tests conducted on six women before and during therapy with a contraceptive pill containing estrogen and progesterone. At the same time PHLA was depressed while taking the pill. Further experiments were conducted to provide an explanation for decreased PHLA without a change in fat tolerance. When increasing doses of heparin were administered, decreased PHLA during therapy was attributed to decreased LPL release.

More recent reports have separated the LPLA and hepatic lipase activity in post-heparin plasma. Glad et al. (19) measured PHLA, hepatic lipase activity and LPLA after increasing amounts of heparin were injected in women on a combined oral contraceptive pill. They found no differences in LPLA but did measure decreased hepatic lipase activity in all but one heparin dose in women taking the pill. Total PHLA was significantly reduced at the higher heparin doses, which were equivalent to the middle of the dosage range used by Hazzard et al. (18). They concluded the releasable pool of hepatic lipase is decreased by a combined oral contraceptive. Applebaum et al. (20) reported a high correlation (0.969) between hepatic triglyceride lipase and PHLA in a paired study of women taking or not taking ethinyl estradiol for 2 weeks. Extrahepatic LPLA was not significantly changed. A large variation but no significant change in adipose tissue LPLA was found. They concluded the decreased PHLA was due to the effect of estrogen on hepatic triglyceride lipase. Neither total PHLA nor hepatic lipase activity were correlated with the increase in serum triglycerides. From the changes in hepatic lipase and results of Hazzard et al., it is possible that decreased PHLA during estrogen therapy is due to hepatic lipase resistance to heparin release.

The influence of sex steroid hormones on adipose tissue LPLA has been investigated. Hamosh and Hamosh (21)

injected ovariectomized rats for 7 days with 17-β-estradiol or progesterone. Adipose tissue LPLA was decreased in estradiol treated rats while no change was found in progesterone treated rats compared to ovariectomized rats. Plasma triglycerides were significantly elevated in estradiol treated rats. There were no differences in heart or lung LPLA. Kim and Kalkhoff (22) injected rats with estradiol and/or progesterone for 21 days. They found an increase in triglyceride entry into the plasma in estradiol and estradiol plus progesterone treated rats which correlated with the hypertriglyceridemia. The lipase activities which they reported (Table 1) did not correlate with the serum triglyceride concentration.

Table 1. Sex Steroid Hormone Effects on Lipase Activity And Plasma Insulin

	Progesterone	Estrogen	Estrogen-and-Progesterone
Total PHLA	↑	↓	↓
Hepatic Lipase	↑	↓	↑
Adipose LPL (per ug DNA)	↑	↓	↑
Plasma Insulin	↑	↑	↑

From Kim and Kalkhoff (22).

Kim and Kalkhoff (22) concluded that sex steroids primarily influence plasma triglyceride concentrations by

their action on hepatic synthesis. It is difficult to interpret the sex steroid effects on hepatic lipase and adipose LPL from their data. Total PHLA was decreased by the E+P treatment, yet hepatic lipase and the tissue lipases, adipose and mammary gland, were increased. Unless a significant amount of tissue lipase other than parametrial adipose tissue and mammary gland were contributing to the PHLA, the results are contradictory.

Two reports have used triglyceride turnover rate, determined during a steady state, as a measurement of triglyceride production. Kekki and Nikkila (23) measured triglyceride turnover by injecting ^{3}H-glycerol into women taking a combined oral contraceptive and compared the rates to those of women who had never taken oral contraceptive pills. Both the triglyceride concentration and production rate were significantly greater in women taking the oral contraceptive. Data from the control group were used to develop an equation relating triglyceride concentration to the turnover rate in order to predict the triglyceride concentration of women taking the pill from their turnover rates. The increase in triglycerides in women taking oral contraceptives was not as great as would be expected from the twofold increase in production rate. Enzyme kinetic analysis of the data resulted in a calculated K_m for the treated group which was significantly less than the control. The K_m was used as a measure of triglyceride removal

efficiency. Since the substrate concentration at which the turnover was ½ of V_{max} was lower for the treated group, the removal efficiency was said to be greater. They concluded that women taking estrogen and progesterone had an increase in the production of triglycerides and greater removal efficiency from the plasma. Kissebah et al. (24) compared women taking a combined preparation, or a progestin, or an estrogen to women who had never taken oral contraceptive steroids. The results are shown in Table 2.

Table 2. Oral Contraceptive Effect on Factors Determining Serum Triglyceride Concentrations

Treatment	Serum Triglyceride Concentration	Turnover Rate	K_m	PHLA	Intralipid Clearance
Estrogen + Progesterone	↑	↑	↓	—	↑
Progesterone	↓	—	↓	↑	↑
Estrogen	↑	↑	—	↓	—

From Kissebah et al. (24).

Triglyceride turnover results (measured after infusion of ^{14}C-palmitate) were similar to those of Kekki and Nikkila (23). Clearance of exogenous triglycerides was measured by the rate of Intralipid removal. The clearance rate was higher in women taking progestins alone or in combination with estrogen. The data indicate that estrogen

mainly causes an increased production of triglycerides while progestin results in an increased triglyceride removal efficiency.

The chicken has been used as a model to study hepatic lipid synthesis during estrogen induced hyperlipidemia. Chickens are an appropriate model for studying hypertriglyceridemia induced during pregnancy since plasma triglyceride and cholesterol levels and lipoprotein composition are similar to humans (Kudzma et al. 25). They also measured changes in lipoprotein composition after 18 days of diethylstilbestrol (DES) treatment. Chickens treated with DES had a dose related increase in plasma triglyceride and cholesterol concentrations found primarily in the VLDL, similar to women taking estrogen (26). ^{14}C-acetate incorporation into triglycerides and phospholipids of liver slices from DES treated chickens was greater than control birds. No differences were found in the incorporation into cholesterol or free fatty acids. It was not clear if any differences in pools of endogenous acetate were considered. The chicks were fasted for one hour before killing. Coleman et al. (27) measured the enzymes of triglyceride synthesis in chicks injected with 2 mg DES for 5 days. Fatty acid CoA ligase, glycerol-3-phosphate acyltransferase and diacyl glycerol acyltransferase activities per liver were increased 230 to 300% of the activity of control chicks. Specific activities of these enzymes,

however, were not different with treatment. The increase in total activity did not seem to be a general effect due to increased protein synthesis but rather to increased synthesis of these enzymes since diacylglycerol choline phosphotransferase and succinic dehydrogenase, a mitochondrial marker activity, were increased only 35% and 24% respectively. Specific activity of these two enzymes decreased slightly. Chan et al. (28) found an increase in mRNA for one of the apoproteins of VLDL after a single injection of 2.5 mg of DES. In another report (29), they measured VLDL production in vitro and in vivo after a single injection of estrogen (1 mg) or estrogen (1 mg) plus progesterone (2 mg). Plasma VLDL concentration increased to a maximum at 48 hours after the injection of estrogen. Estrogen or estrogen plus progesterone treatment also increased VLDL synthesis rates in liver slices. Nafoxidine-HCl, an estrogen antagonist, suppressed VLDL production. The work in chickens indicates that estrogen promotes VLDL synthesis in the liver by increasing enzymes for triglyceride and apoprotein synthesis.

From the literature, it seems that both hepatic triglyceride synthesis and decreased triglyceride removal from the blood are involved in hypertriglyceridemia during pregnancy. The reports indicate that sex steroids have an effect on enzymes involved in both mechanisms. Estrogen may or may not be affecting LPLA. It is difficult to separate

the hormonal effects from the dietary state of the animal in the investigations so far. Estrogen does have an effect on enzymes involved in triglyceride synthesis. The influence of estrogen on hepatic synthesis when substrate is limited to the non-pregnant level of food intake has not been considered.

Prolactin has been considered a hormone primarily involved in lactation, but increased prolactin concentrations seen just prior to parturition and the stimulation of prolactin secretion by estrogen, are reasons to consider prolactin when hormonal influences on blood triglyceride concentrations during pregnancy are studied. The experiments in this thesis were conducted to: (1) determine the mechanism of the increase in blood triglycerides during pregnancy, (2) separate the hormonal and dietary influence on hypertriglyceridemia during pregnancy, and (3) determine if prolactin has any effect on hypertriglyceridemia during pregnancy.

MATERIALS AND METHODS

Animals and Study Design

For all experiments, Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) were housed in a temperature-humidity controlled room lighted from 7 AM to 7 PM and were fed the same diet.[1]

Experiment I was conducted to study the effects of physiological doses of estrogen and progesterone on plasma triglycerides and adipose tissue LPLA in ovariectomized rats. Rats weighing 190-210 g were kept individually in hanging wire cages. The above diet was fed and food intake was recorded starting three days after ovariectomy. Treatments were started one week after the operation. Daily subcutaneous injections of the following were given for 21 days between 9 AM and 10 AM: (1) 0.1 ml of sesame oil (control, C); (2) 2.0 mg progesterone (P); (3) 1.0 ug β-estradiol-3-benzoate (E); (4) 2.0 mg progesterone and 1.0 ug β-estradiol-3-benzoate (E+P) (Sigma Chemical Co., St. Louis, MO). This dosage of estrogen and progesterone

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[1]The diet contained (metabolizable energy basis): 20% casein, 59% cornstarch, 1% glucose, 5% corn oil, and 15% lard. Commercial mineral and vitamin mixes were added to meet NRC requirements (See Appendix A).

causes mammary growth similar to that observed during pregnancy (30). On days 12, 19, and 21, six rats from each group were decapitated, blood was collected in heparinized tubes and the parametrial adipose tissue was removed and placed in ice-cold 0.15 M NaCl.

In Experiment II, the effect of prolactin and inhibition of its secretion on plasma triglycerides and adipose tissue LPLA was studied. Since estrogen stimulates prolactin secretion in rats, the animals were ovariectomized to control endogenous estrogen levels. Rats were housed individually in hanging wire cages and food intake was measured. Groups I, III, IV, and V received 1 ug E and 2.0 mg P in 0.1 ml sesame oil and Group II received 0.1 ml sesame oil daily by subcutaneous injections starting one week after ovariectomy. Rats were injected twice the day before and once the morning of killing with: Group I and II--0.2 ml 20% ethanol in saline; Group III--0.1 mg 2-bromo-α-ergocryptine (CB-154), an inhibitor of prolactin secretion, (provided by Sandoz, Inc., E. Hanover, NJ); Group IV--0.1 mg CB-154 and 25 I.U. of prolactin (NIH-P-B5, provided by National Institute of Arthritis, Metabolism and Digestive Diseases) and Group V--25 I.U. of prolactin. This dosage of CB-154 and prolactin is twice the amount used to inhibit and restore lactation in the rat (personal communication from D. E. Bauman). The CB-154 and prolactin were first dissolved in ethanol and a drop of 1 N NaOH. NaCl (0.15M)

was added to give a final concentration of 20% ethanol. One-half of the total dose was injected subcutaneously twice the day before and once the morning of killing on days 8 and 21. Twelve-hour fasted rats were decapitated, blood was collected in heparinized tubes and parametrial adipose tissue was removed and placed in ice-cold 0.15 M NaCl. Livers for triglyceride synthesis rate determinations were removed and placed in ice-cold tris-sucrose buffer (pH 7.2; 30 mM tris, 0.3 M sucrose, 1 mM GSH, 1 mM EDTA).

Experiment III was conducted to investigate the effects of prolactin and inhibition of its secretion on triglyceridemia during pregnancy. Sprague-Dawley rats (200-220 g) were mated. The day sperm were seen in the vaginal smear was designated day 1 of pregnancy. Food intake was recorded and restricted after day 12 to the amount consumed by 12-day pregnant rats in order to control substrate availability for triglyceride synthesis. Twenty-four rats were assigned to 3 treatment groups: (1) 0.2 ml 20% ethanol in saline, (2) 0.1 mg CB-154 or (3) 0.1 mg CB-154 and 25 I.U. of bovine prolactin. The injections were given subcutaneously between 9 AM - 11 AM and 5 PM - 6 PM the day before killing and between 9 AM - 11 AM the day of killing. Rats were fasted 12 hours and killed 2 hours after the morning injection on days 0, 12, 19, and 21 of pregnancy. Virgin rats were on the diet at least 2 weeks prior to killing.

After decapitation, blood was collected in heparinized tubes and parametrial adipose tissue, placentas, and the left mammary gland were removed and placed in ice-cold 0.15 M NaCl. Uterine contents of 12-day pregnant rats were scraped out of the uterine lining and analyzed as placentas. Livers were removed and placed in ice-cold tris-sucrose buffer.

Tissue Preparation

Adipose tissue and placentas were blotted, weighed and homogenized in 2 volumes per gram of tissue with 0.025 N $(NH_4)_2SO_4$ - 0.15 M NaCl; pH 8.6 containing 1 unit of heparin per ml. Acetone-ether powders were prepared as described by Robinson (31) and modified by Hamosh et al. (6). Powders were stored in a dessicator at 4° C until analyzed for LPLA. Mammary glands were blotted dry, weighed, sliced with a scalpel, frozen on dry ice and ground with dry ice in a motor-driven mill (Quaker City Mill, Philadelphia, PA). The frozen particles were scraped into acetone and acetone-ether powders were prepared.

Livers were homogenized in 2 volumes of ice-cold tris-sucrose buffer for 30 seconds with a Lourdes homogenizer (Vernitron Medical Products, Inc., Carlstadt, NJ) followed by 5 passages on a teflon pestle tissue grinder. The supernatant formed by centrifuging for 20 minutes at 10,000 x g at 4° C was frozen in aliquots for enzyme and protein analysis.

Assay Methods

Plasma was analyzed for triglycerides according to the method of Biggs et al. (32). Free fatty acids were extracted according to Dole (33) and analyzed by Itaya and Ui's method (34).

Adipose tissue, placentas and mammary glands were analyzed for LPLA using Corey and Zilversmit's method (35). Adipose tissue and mammary gland powders were homogenized in 3 volumes per gram of tissue (wet weight) with 0.025 N $(NH_4)_2SO_4$ - 0.15 M NaCl; pH 8.6 with 1 unit of heparin per ml in ground glass homogenizing tubes. Placentas were homogenized with 6 volumes per gram of tissue. Activity was linear for 60 minutes of incubation at 37° C. All tissues of one experiment were assayed at the same time using the same rat serum as a source of apoproteins to avoid variations due to activating factor(s). Aliquots of the extracts containing fatty acids were neutralized before liquid scintillation counting.

Liver triglyceride synthesis rates were measured by $^{14}C(U)$-*sn*-glycerol-3-phosphate (New England Nuclear, Boston, Mass.) incorporation into glycerides (Bennink, 36). Optimum pH was 7.2 and incubations were for 45 minutes at 37° C.

Other methods used were: DNA in adipose tissue--Setaro and Morley (37); DNA in placenta and mammary gland--Dische (38); and protein--Lowry (39).

One-way analysis of variance was used for Experiment I and two-way analysis of variance was used for Experiments II and III to determine treatment differences. When the F value was greater than the critical value, Tukey's, Student's t or Scheffe's test were used where indicated to compare the means (40).

RESULTS

Effect of Sex-Steroid Hormones on Plasma Triglyceride Concentration and Adipose LPLA

Rats treated with estradiol (E) or estradiol and progesterone (E+P) had significantly greater ($P < .05$) plasma triglyceride concentrations than the control group (Table 3). Adipose tissue LPLA was lower for E and E+P treated groups when expressed on a total tissue basis, but no differences were found when the activity was based on DNA content. The E and E+P treated groups also ate significantly less than the control group and were significantly smaller ($P < .05$) (Appendix B-1). The adipose tissue wet weight of the E treated group was significantly less than the control group, while the E+P treated group was no different from the control group. Progesterone alone did not significantly affect the plasma triglyceride concentration or adipose tissue LPLA compared to the control values. Plasma-free fatty acid concentrations were not different in any treatment group.

Table 3. Sex Steroid Hormone Effects on Plasma Triglyceride Concentration and Adipose Tissue Lipoprotein Lipase Activity[1]

	Plasma Triglyceride (mg/100 mls)	LPLA (nmoles oleate) (tissue - min.)	LPLA (nmoles oleate) (ug DNA - min)	Free Fatty Acids (uEq/l)
Sesame Oil	49	82	0.61	672
Progesterone	60	105	0.57	687
Estrogen	119*	44*	0.46	782
Estrogen + Progesterone	113*	49*	0.80	597
s.e.[2]	11	13	0.14	53

[1]An * denotes a significant difference from the control group ($P < .05$) using Student's t-test.

[2]s.e. is the pooled standard error of the treatment means, 18 animals per treatment.

Effect of CB-154 and/or Prolactin in Ovariectomized Rats

Inhibition of prolactin secretion or the addition of prolactin did not affect plasma triglyceride concentration, adipose tissue LPLA or liver triglyceride synthesis rate after 8 or 21 days of injection (Table 4). Rats treated for 8 days had lower plasma triglyceride concentrations and lower adipose tissue LPLA than rats treated for 21 days, however the trend in differences between groups was the same. The animals given estrogen and progesterone had higher, but not significantly different, plasma triglycerides than those given sesame oil. The fasted state of these animals reduced the magnitude of the differences found in the first experiment. They were significantly lighter and ate significantly less than rats injected with sesame oil (Appendix B-2).

Triglyceridemia Changes During Pregnancy

Pregnant rats had significantly greater plasma triglyceride concentrations on days 19 and 21 compared to day 12 and non-pregnant rats when food intake was controlled (Table 5). The in vitro removal capacity of the tissues did not entirely reflect the differences in plasma triglyceride concentration. Adipose tissue LPLA (per total tissue) was significantly lower on days 12, 19, and 21 of

pregnancy than LPLA of non-pregnant rats. Liver triglyceride synthesis rates were not different at any time. When LPLA was expressed on a DNA basis, period of gestation did not affect LPLA except for adipose tissue. Adipose LPLA progressively declined from the non-pregnant state to day 21 of pregnancy, but 12-day pregnant rats were not significantly different from non-pregnant or 19-day pregnant rats.

Effect of CB-154 and Prolactin on Pregnant Rats

Inhibiting prolactin secretion with CB-154 increased plasma triglyceride concentrations of rats pregnant 19 or 21 days but had no effect on non-pregnant or 12-day pregnant rats. When prolactin and CB-154 were co-injected, plasma triglyceride concentrations returned to the control level. Neither treatment produced changes in LPLA or liver triglyceride synthesis rates (Table 6).

Table 4. Effect of Inhibition or Addition of Prolactin on Enzymes Affecting Plasma Triglyceride (TG) levels in Fasted Ovariectomized Rats

	Plasma Triglyceride Concentration (mg/100 mls)		Adipose LPLA (nmoles oleate)/(tissue-min)		Adipose LPLA (nmoles oleate)/(ug DNA-min)		TG Synthesis (nmole TG)/(liver-min)	TG Synthesis (nmole TG)/(mg protein-min)
Treatment[1]	8[2]	21	8	21	8	21		
E+P Saline	32	47	70	111	0.48	1.9	532	0.36
S.O. Saline	22	34	69	123	0.54	1.9	478	0.39
E+P CB-154	35	48	58	127	0.37	1.3	483	0.43
E+P CB-154 Prl	38	43	64	99	0.55	1.2	539	0.49
E+P Prl	33	46	96	120	0.60	1.2	531	0.51
s.e.[3]	6		18		0.23		33	0.07

[1]E+P - 0.1 ug β-estradiol-3-benzoate and 2.0 mg progesterone; S.O. - 0.1 ml sesame oil; CB-154 - 0.2 mg CB-154; Prl - 50 I.U. prolactin.

[2]Values of rats treated for 8 or 21 days are listed separately when significantly different from each other.

[3]s.e. is the pooled standard error of the treatment means, 6 animals per treatment per time of treatment.

Table 5. Changes in Enzyme Activities Affecting Plasma Triglyceride (TG) Concentrations of Fasted Pregnant Rats[1,2]

Day	Plasma TG[3]	Adipose Tissue LPLA[4]		Mammary Gland LPLA[5]		Placental LPLA[5]		Liver TG Synthesis[6]	
0	35[a]	125[a]	2.7[a]	3[a]	1.3[a]	---		217	0.23
12	39[a]	64[b]	1.7[ab]	11[a]	1.7[a]	6[a]	5.1[a]	185	0.17
19	93[b]	70[b]	0.8[bc]	58[b]	3.7[b]	172[b]	10.4[b]	225	0.18
21	101[b]	47[b]	0.5[c]	45[b]	2.9[b]	164[b]	10.4[b]	208	0.17
s.e.[7]	6	12	0.3	5	0.3	9	1.2	35	0.03

[1]Food was restricted after day 12 to the amount consumed by 12-day pregnant rats.

[2]Values with different superscripts in the same column are different ($p < .05$) as determined by Tukey's test.

[3]mg/100 ml.

[4]nmoles oleate/tissue-min; nmoles oleate/ug DNA-min.

[5]nmoles oleate/tissue-min; nmoles oleate/mg DNA-min.

[6]nmoles of TG/liver-min; nmoles TG/mg protein-min.

[7]s.e. is the pooled standard error of the means; 15-19 rats per group.

Table 6. Effect of Reduced Prolactin Secretion and Exogenous Supplementation of Prolactin on Triglyceridemia During Pregnancy

	Plasma TG[1]		Adipose Tissue LPLA[2]		Mammary Gland LPLA[3]		Placental LPLA[3]		Liver TG Synthesis[4]	
Treatment[5]	0-12[6]	19-21								
Saline	35[a]	79[a]	79	1.8	32	2.7	121	8.7	211	0.19
CB-154	33[a]	125[b]	82	1.3	32	2.5	130	9.7	217	0.19
CB-154 Prl	42[a]	84[a]	65	1.1	26	1.9	104	8.3	200	0.19
s.e.[7]	7		10	0.3	4	0.3	23	1.2	30	0.03
n	12		24		20-22		16-18		23-24	

[1]mg triglyceride/100 mls.

[2]nmoles of oleate/tissue-min; nmoles oleate/ug DNA-min.

[3]nmoles oleate/tissue-min; nmoles oleate/mg DNA-min.

[4]nmoles triglyceride/liver-min; nmoles triglyceride/mg protein-min.

[5]Saline - 0.2 mls; CB-154 - 0.2 mg CB-154; Prl - 50 I.U. prolactin.

[6]The effect of the treatment on triglycerides was found only on days 19 and 21 of gestation, so values for days 0 and 12 were grouped together and statistically analyzed separate from days 19 and 21. Values with different superscripts are significantly different ($p < .05$) by Scheffe's test.

[7]s.e. is the pooled standard error of the means for the three treatments.

DISCUSSION

These studies were conducted to determine the mechanism of hypertriglyceridemia during pregnancy. It is generally recognized that food intake increases during the last half of pregnancy simultaneously with increases in serum triglyceride concentrations (4,5,7). In this study, when rats pregnant 19 and 21 days were restricted to energy consumption equal to 12 day pregnant rats, elevated serum triglyceride concentrations were still found (Table 3), indicating that more than food intake is involved in hypertriglyceridemia during pregnancy.

In the restricted energy state, increased substrate for liver triglyceride synthesis could come from free fatty acids hydrolyzed from triglycerides in adipose tissue. An increase in plasma free fatty acids during pregnancy has been reported (41). Free fatty acids may be released from adipose tissue in response to placental lactogen, which increases during pregnancy (42). The free fatty acids could then be taken up by the liver and re-esterified to glycerol-3-phosphate to form triglycerides. In this study, however, liver triglyceride synthesis rates were not increased (Table 5).

Others have reported that elevated serum triglycerides result from increased synthesis during pregnancy. Increased triglyceride mobilization was found during pregnancy when based on the whole rat (5). However, no differences were found when the mobilization was based on unit body size, suggesting that the increase is related to proportional increases in body tissues such as the liver during pregnancy. Increased fatty acid synthesis has also been reported in the fed pregnant rat (10). The increased synthesis was measured by ^{14}C-acetate incorporation into triglycerides and did not account for endogenous pools of acetate, however.

Effects of hormones on triglyceride synthesis have shown that estrogen increases VLDL synthesis in the chicken (28,29), triglyceride mobilization in women taking oral contraceptive pills (23,24), and triglyceride entry into the blood of ovariectomized rats (22). No hormonal effects on triglyceride synthesis were found in this study (Table 4).

The lack of difference in triglyceride synthesis rates in this study may indicate that substrate availability is more important in determining the rate of triglyceride synthesis by the liver during pregnancy. Ad libitum fed pregnant rats have higher serum triglyceride concentrations (4,5,7,Appendix B-3) than restricted rats in this study. When substrate supply is limited, increased blood triglyceride concentrations may result from the estrogenic effect on

triglyceride removal. The interaction of hormones such as estrogen and the increased substrate supply during the latter half of pregnancy may further enhance the hypertriglyceridemia.

Changes in triglyceride removal capacity of LPL in some tissues during pregnancy have been reported. The adipose tissue LPLA was found to decrease late in pregnancy in this study (Table 5) and others (5,6,7). A decrease was seen as early as day 12 of pregnancy when expressed on a total tissue basis, however the activity per ug of DNA on day 12 was not different from non-pregnant rats in this investigation. Others (5,6) have reported no change in adipose LPLA (per g of tissue) until after day 12 of pregnancy.

Hypertriglyceridemia and decreased adipose tissue LPLA found in ovariectomized rats treated with estradiol or estradiol and progesterone (21, Table 3), suggests that the contribution of estradiol to increased serum triglycerides during pregnancy is through the hormone's effect on triglyceride removal by adipose tissue LPL. The mechanism of estradiol's effect on triglyceride removal from blood is not clear. One possibility is a direct effect of estradiol on the tissue to stimulate synthesis of LPL. The general mechanism of estrogens' action on tissues is through the formation of an estrogen-protein receptor complex in the cytosol which moves to the nucleus and interacts with chromatin to initiate mRNA synthesis. Another possibility is through the

hormones stimulation of apoprotein synthesis in the liver (28). At least one apoprotein (apo C-II) has been found to activate LPL (43,44) and is a necessary factor in the assay system for the enzyme. Other apoproteins (apo C-III's) have been reported to inhibit LPL (45). When serum from estrogen plus progesterone treated rats was used as a source of activating factor(s), adipose tissue LPLA was depressed compared to control serum (46,47). The effect of apoproteins has been demonstrated on heart (48,49) and adipose LPL (50). Since both activators and inhibitors are present in serum, the ratio of the apoproteins would be the determining factor on enzyme activity. The mechanism seems plausible, however, Hillman et al. (51) reported no changes in the ratio of apo C-II and apo C-III in VLDL of pregnant women.

From the increased activity of mammary (6, Table 5) and placental LPLA (52, Table 5) on days 19 and 21 of pregnancy, it would seem that either apoproteins are not effectors of the enzyme in these tissues as they are in adipose tissue or that other hormonal interactions are influencing the activity. Prolactin was found to increase mammary gland LPLA and decrease adipose tissue LPLA of lactating rats with no effect on the plasma triglyceride concentration (53). During pregnancy serum prolactin concentrations are low until just before parturition (54). It would seem possible that the large increase in mammary gland LPLA found

just prior to parturition (6, Table 5) could be due to prolactin. However, in this study, prolactin or inhibition of its secretion did not affect LPLA of adipose tissue, mammary gland or placenta. Plasma triglyceride concentrations were increased on days 19 and 21 when prolactin secretion was inhibited by CB-154, however, the increase could not be attributed to changes in adipose, mammary or placental LPLA or liver triglyceride synthesis rates (Table 6).

The role of placental lactogen, which has some biological effects similar to prolactin, has not been fully investigated due to the difficulty in obtaining a pure source of the hormone.

More research on placental lactogen and other factors that could potentially affect the blood triglyceride concentration during pregnancy needs to be conducted in order to fully understand the mechanism of hypertriglyceridemia.

SUMMARY

The influence of hormones and increased food consumption on hypertriglyceridemia during the last half of pregnancy was investigated in this study. Plasma triglyceride concentrations were increased even when food intake was restricted after day 12 to the amount consumed by 12-day pregnant rats. Adipose tissue LPLA decreased; placental and mammary gland LPLA increased and liver triglyceride synthesis rates did not change when food intake was controlled during pregnancy. From the effects of estradiol on the ovariectomized rat adipose LPLA, estradiol is responsible for the decreased adipose LPLA in pregnant rats. Prolactin decreases plasma triglyceride concentrations late in pregnancy, but the enzyme activities measured did not correlate with the changes in plasma triglyceride concentration which occurred when prolactin secretion was inhibited and exogenous prolactin was administered.

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APPENDIX

APPENDIX A

Diet Composition

Basal Mix		Diet	
Casein	263 g	Basal Mix	396 g
Mineral Mix[1]	40 g	Cornstarch	511 g
Vitamin Mix[2]	20 g	Fat (lard)	64 g
Cellulose	40 g		
Corn Oil	22 g		
Glucose	6 g		
DL-methionine	3 g		
Choline Chloride	2 g		
	396 g		

[1]Mineral Mix No. 4164 (Teklad Test Diets, Madison, Wisconsin).

[2]Vitamin Supplement 23430 (U. S. Biochemical Corp, Cleveland, Ohio).

APPENDIX B

Table B-1. Food Consumption, Body Weights and Adipose Tissue Wet Weights of Estradiol and/or Progesterone Treated Ovariectomized Rats[1]

	Food Consumption	Body Weight	Adipose Tissue
	(g)	(g)	(g)
Sesame Oil	19	297	1.9
Progesterone	19	295	1.7
Estradiol	16*	249*	1.2*
Progesterone + Estradiol	16*	261*	1.4
s.e.[2]	0.5	4	0.1

[1] An * denotes a significant difference from the sesame oil (control) treated group ($p < .05$) by Tukey's test (18 rats per group).

[2] s.e. is the pooled standard error of the means.

Table B-2. Food Consumption, Body Weights and Adipose Tissue Wet Weights of CB-154 and/or Prolactin Treated Ovariectomized Rats[1]

Treatment	Food Consumption[2] (g)	Body Weight (g)	Adipose Tissue (g)
E+P Saline	7[a]	244[a]	2.6
S.O. Saline	9[b]	264[b]	2.7
E+P CB-154	7[a]	236[a]	2.4
E+P CB-154 Prl	6[a]	242[a]	2.7
E+P Prl	7[a]	245[a]	2.9
s.e.[3]	0.4	3	0.3

[1]Values with different superscripts are different ($p < .05$) by Tukey's test (12 rats per group).

[2]During the 24 hours before being killed, the rats consumed the amount indicated during the first 12 hours and were fasted for the second 12 hours.

[3]s.e. is the pooled standard error of the means.

Table B-3. Plasma Triglyceride Concentration (mg/100 ml) of Energy Restricted Fasted and Ad Libitum Fed Pregnant Rats[1,2]

Day	Ad Libitum Fed	Energy Restricted
0	27[a]	33[a]
12	77[b]	38[a]
19	124[c]	74[b]
21	111[c]	86[b]
s.e.[3]	13	6
n	5	6

[1]Values in the same column with different superscripts indicate a significant difference ($p < .05$) by Tukey's test.

[2]Ad libitum fed rats had access to the same diet as the energy restricted rats until the time they were killed.

[3]s.e. is the pooled standard error of the means.