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Lipoprotein Lipase Activity of Rat and Human Placenta.* (30162)

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The enzyme lipoprotein lipase (LPL), also known as clearing factor lipase, which hydrolyzes the triglyceride moieties of circulating low density lipoproteins and chylomicrons, has been reported to be present in a number of tissues and organs(1-3). It is released into the circulation following intravenous injection of heparin, heparinoids, and other negatively charged polyvalent anions(3). In the rat, the highest activities of the enzyme have been reported to occur, normally, in heart and adipose tissue(1,2). Unusually high activities also appear in the mammary gland of the guinea pig during lactation(4,5).

Because of the extreme rapidity with which the enzyme appears in the circulation *in vivo* following injection of heparin, or of high molecular weight dextran, which does not leave the circulation readily, and because of evidence suggesting that the triglycerides of chylomicrons and low density lipoproteins are hydrolyzed prior to their passage from the blood into the tissues, it has been postulated that LPL is located in the walls or on the luminal surfaces of capillaries(3,6). It has also been proposed that the role of LPL is to provide tissues with free fatty acids (FFA) that may serve as metabolic substrates for energy production or for synthesis of storage fat and other specific lipids. Thus, it has been observed that LPL activity is increased in heart under conditions promoting metabolic activity (energy production and work) (7), in adipose tissue, in physiological states promoting fat storage(3), and in mammary gland, in the situation in which milk fat is synthesized in large quantity(4,5).

Inasmuch as the placenta is a highly vascular organ and therefore may present considerable tissue in which LPL is localized,

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and since the need of the growing fetus for fatty acid utilization may be high, it was considered possible that for either or both of these reasons, the placenta might possess relatively high LPL activity. The following experiments were therefore carried out to test this hypothesis.

Materials and methods. Female Holtzman albino rats, 250-300 g and not pregnant, as well as similar rats 18-21 days pregnant, as determined by vaginal smears, were anesthetized with pentobarbital administered intraperitoneally, and exsanguinated. The hearts, portions of abdominal adipose tissue, and, in the case of the pregnant rats, the embryos, were removed, and the placentas were dissected away from the fetuses. Only the ventricles of the hearts were used. The tissues were rinsed in cold saline, immediately placed on saline-moistened filter paper in Petri dishes kept on ice, and transferred to a cold room (1-3°C) where 30 or 60 mg sections were removed, blotted with filter paper, and weighed on a torsion balance. Sections of placenta were taken from the fetal side.

Each weighed section was placed into 6 ml of medium, with or without LPL inhibitor, in a glass homogenizing tube surrounded by crushed ice. The medium was composed of 0.6 ml of a 5% coconut oil emulsion (obtained by diluting Ediol,‡ a commercial coconut oil emulsion, with Krebs-Ringer bicarbonate buffer solution at pH 7.4), 2.7 ml of serum obtained fresh from dogs fasted 16-24 hrs, and 2.7 ml of 5% bovine albumin in the above buffer solution. The powdered albumin (fraction V, Pentex, Inc.) was washed with acidified 4:1 isopropyl alcohol-heptane, and then with isopropyl alcohol, to remove FFA prior to its solution in buffer. Each piece of tissue was homogenized in its medium with the aid of a motor driven Teflon pestle. The tubes were than taken to the laboratory, where, following the removal of 1 ml aliquots of homogenate for initial determinations of FFA, their contents were transferred to 25 ml Erlenmeyer flasks and incubated for 30 or 60 minutes at 37°C in a Dubnoff metabolic incubator, with shaking, under an atmosphere of 95% O_2 and 5% CO_2. At the end of the incubation periods, 1 ml aliquots of homogenate were again removed for final FFA determinations. Control flasks containing medium, but no tissue, were simultaneously incubated in order to correct for any FFA formed during the incubation in the medium alone. FFA concentrations were determined by the method of Dole(8) using Nile Blue A as the indicator. The initial FFA concentration was subtracted in each case from the post-incubation one, and the lipase activity of each sample expressed as microequivalents of FFA formed per mg of tissue nitrogen during the period of incubation. In all cases, the LPL activities of 2 or 3 samples of each tissue were determined, and the average of these determinations considered as the value representing that tissue.

For determination of LPL activities of rat tissues, 30 mg sections were used, and the homogenates incubated for ½ hour. Because of the smaller LPL activities manifested by the human placentas, it was found desirable to use 60 mg sections and to incubate homogenates of these for 1 hour, to obtain more accurate results. In all experiments where the effects of inhibitors were assessed, 60 mg sections were also used and incubations carried out for 1 hour, since it was found that this procedure also permitted a better evaluation of the degree of inhibition exerted. The LPL inhibitors employed were protamine sulfate (5 mg/ml), NaCl (1 M) and diisopropylfluorophosphate (DFP) (2×10^{-5} M).

The human placentas were obtained from neighboring hospitals within an hour after delivery. They were placed in a refrigerator by the hospital personnel, and were brought to the laboratory in vessels kept on ice, where sections were removed from the half nearest the fetus and treated as already described.

Fifty to 70 mg sections of all hearts and placentas, and 200 mg sections of the adipose tissues were also removed for tissue nitrogen determination. The sections were digested with sulfuric acid and hydrogen peroxide, then diluted and directly Nesslerized.

In early experiments, NH_4Cl-NH_3 buffer,

‡ We would like to thank Dr. Leon Freeman, Riker Laboratories, Inc., Northridge, Calif. for kindly supplying us with Ediol.

TABLE I. Comparison of LPL Activities of Tissues of Pregnant and Non-Pregnant Female Rats.

Tissue	Pregnant No. of rats	Pregnant μEq FFA/mg tissue N*	Non-pregnant No. of rats	Non-pregnant μEq FFA/mg tissue N*	P
Heart	9	1.60 ± .186	11	1.98 ± .190	>.1
Adipose	6	20.3 ± 3.00	6	17.2 ± 2.23	>.1

* All tissues incubated for ½ hr. ± = standard error of mean.

at pH 8.5, was used instead of the Krebs-Ringer bicarbonate buffer at pH 7.4, since it has been reported that pH 8.5 was optimum for LPL activity of extracts of acetone powders of tissues(1). Others have found the optimal pH for LPL activity in tissue homogenates to be near 7.4, however(9). Inasmuch as it was found, in the case of our homogenates, that the results obtained with both buffers were indistinguishable, the results reported here are those obtained with the bicarbonate buffer only.

Results. To test for the adequacy of the procedure for assay of LPL activity, different quantities of each type of tissue were homogenized in the usual volume of medium and incubated for periods of ½ and 1 hour. In all instances, a straight line relationship was found to exist between the quantities of FFA formed and those of lipase present in the incubation flasks. These relationships for rat heart, placenta, and human placenta, for the 1-hour period of incubation, are shown in Fig. 1.

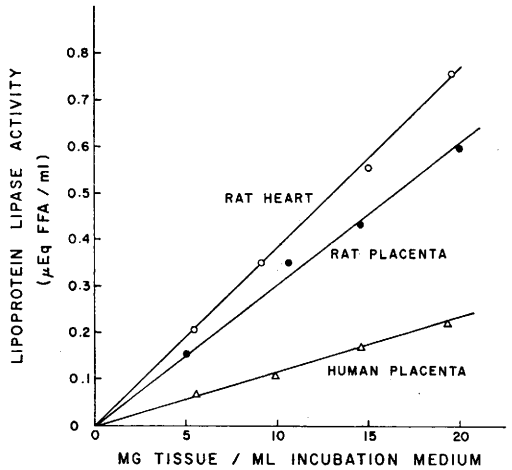

FIG. 1. Relationship between lipoprotein lipase concentration in medium and free fatty acids released during 1 hr of incubation.

When aliquots of homogenates were removed from the incubating flasks after different intervals of time, it was found that the rate of FFA formation was constant during the first half hour, but fell off somewhat during the second half hour. For this reason, it was considered preferable to use a ½-hour period of incubation when possible.

In order to know whether the LPL activity of rat placenta could be compared with the LPL activities of other tissues, in the non-pregnant as well as the pregnant rat, it was necessary to determine whether the LPL activities of the same tissues were alike in both pregnant and non-pregnant animals. It was observed that there was no significant difference in the LPL activities of heart or of adipose tissue between pregnant and non-pregnant female rats of the same age (Table I).

The LPL activity of rat placenta was found to be as high as that of rat heart, while the LPL activities of both of these tissues were considerably smaller than that of rat adipose tissue, when expressed on a tissue nitrogen basis. The LPL activity of human placenta was significantly lower than that of rat placenta. Although one cannot make an exact comparison in the latter case, the value for human placenta would be a little more than one-half of the value of 0.88 μEq FFA/mg N obtained for 1 hour incubation, as compared with the value of 1.73 μEq FFA/mg N/½ hour incubation for rat placenta (Table II).

To find out whether the lipase activities determined in our assay truly represented LPL activities, lipase activities of the tissue homogenates were determined in the absence of serum and in the presence of LPL inhibitors. It has been shown that LPL activity is markedly reduced in the absence of serum

TABLE II. LPL Activities of Pregnant Rat Placenta, Heart and Adipose Tissue, and of Human Placenta.

Tissue	No. of rats or of human placentas	Lipase activity, μEq FFA/mg tissue N/incubation period*
Rat adipose	32	23.0 ±1.65
" heart	35	1.66 ± .105
" placenta	36	1.73 ± .148
Human placenta	13	.88 ± .136

* All tissue homogenates were incubated for ½ hr except those of human placenta, which were incubated for 1 hr.
± = standard error of mean.

in the assay mixture, or if the fat emulsion has not been previously activated by preincubation with serum(3,10). Certain substances have also been shown to inhibit LPL activity in contrast to pancreatic lipase activity, but the degree of inhibition appears to vary with the type of preparation used(6). In the absence of serum, the lipase activities of the homogenates of placenta were found to be decreased by about 60%. DFP, protamine and 1 M NaCl inhibited lipase activities of all tissues. The degree of inhibition of activity of rat placenta was about the same as that of rat heart and adipose tissue (40-80%) while homogenates of human placenta appeared to be somewhat more inhibited than the homogenates of rat tissues (74-81%) (Table III). If it is assumed that the inhibitors inhibit LPL completely, and that LPL is inactive in the absence of serum or of serum-activated fat emulsion, then our data indicate that at least 50% of the lipase activities assayed were due to LPL.

Since placenta is highly vascular and the organs *in vitro* contain residual blood, the question may be raised as to the possible contribution of residual blood LPL activity to the lipase activity measured. In the post-absorptive state, LPL is absent, normally, from the circulation in man and animals(3). Several determinations were made of lipase activity of the blood of pregnant rats, in our laboratory, and it was found that the activity was small enough so that its contribution to the total activity measured, in sections of placenta, could be entirely neglected. No determinations were made, however, of LPL activity of fetal blood. The placentas were not washed free of blood by perfusion prior to measurement of lipase activity. It has been suggested that the complete washing out of blood would probably destroy all but the most hardy of placental enzymes(11).

Discussion. A large number of enzymes has been reported to be present in the placenta(11). Lipase has been reported to be both absent(12) and present(13,14). Our data indicate that the placenta is also relatively rich in LPL. The lower activity manifested by human, as compared with rat placenta, may be due to one or more causes: 1) a natural difference between the 2 species; 2) the greater delay encountered before assaying the human placental tissue after its removal from the physiological environment; and 3) a difference between LPL activities of placenta during gestation and post partum. With regard to the second point, the instability of LPL in tissue preparations *in vitro*, even at room temperature, is well known. In the case of the lactating mammary gland, it was found that 18 hours after weaning, LPL activity dropped from a high value to zero, and activity in the milk decreased 36% after

TABLE III. Inhibition of Lipase Activities by LPL Inhibitors, and Reduction of Lipase Activities in Absence of Serum.

Tissue	% Inhibition by DFP (2×10^{-5} M)	% Inhibition by protamine (5 mg/ml)	% Inhibition by NaCl (1 M)	% of control activity in absence of serum
Rat adipose	51.0 ± 4.17 (7)	50.3 ± 3.73 (8)	66.4 ± 4.23 (7)	
" heart	42.1 ± 4.77 (9)	53.1 ± 2.86(12)	79.7 ± 2.60 (6)	
" placenta	45.9 ± 5.32 (9)	39.7 ± 3.60(11)	59.5 ± 3.19 (7)	41.5 ± 4.93 (9)
Human placenta	80.9 ± 6.88 (8)	73.9 ± 8.11 (7)	77.8 ± 6.57 (8)	45.7 ±10.90 (4)

± = standard error of mean.
Numbers in parentheses refer to number of rats or of human placentas.
All tissue homogenates were incubated for 1 hr.

3 hours of incubation at 38°C, indicating the instability of the residual enzyme in tissues and body fluids(4). As far as the third possibility is concerned, it has been pointed out that as gestation progresses, various enzymes of the placenta increase and decrease in activity, independently of one another(11). At parturition, lipase activity has been reported to decrease quickly(14). Hence it is possible that LPL activity of the placenta may be lower at the time of delivery than during the latter part of gestation.

The high LPL activity in placenta may be considered in relation to two phenomena: 1) the vascularity of the placenta and 2) the function of this organ. If LPL is indeed located in the walls of all blood vessels, one would expect a highly vascular organ such as placenta to have considerable quantities of this enzyme. In the hemo-chorial placentas of man and rat, in which maternal arterioles deliver blood into sinuses surrounding the villi, the LPL may be predominantly located in the fetal blood vessels of the placenta.

From the functional point of view, whether the LPL is located in the blood vessels or in other tissues of the placenta, it would make good teleological "sense" for the organ to have high LPL activity, since one of its main functions is to transport nutrients from the maternal to the fetal circulation. Although the fetus has been shown to be capable of synthesizing lipids readily, the need of the growing fetus for fatty acids may be great, particularly during the latter part of pregnancy, when the mammalian fetus rapidly builds its stores of body fat(15). Consequently, utilization of fatty acids supplied by the mother may be necessary as well. Such fatty acids may be offered from the maternal circulation in the form of FFA and lipoprotein triglyceride. The well known lipemia of pregnancy may be related to this need(16). The consensus is that small molecules, in general, can cross the placental barrier more readily than larger ones(17). Chylomicrons and large lipoproteins may not be transported across the placenta as readily as fatty acids formed from the former by lipolysis in the placenta *via* LPL. It has been shown in numerous experiments involving the feeding of pregnant animals with "physiological" unsaturated fatty acids, their esters or triglycerides, trans-isomers of unsaturated fatty acids or their triglycerides(18-20), and the feeding or injection of isotopically labeled fatty acids (21-23) that fatty acids do cross the placental barrier. It is of interest to note that evidence has been obtained that maternal circulating phospholipids, avidly absorbed by the placenta, are degraded there without being transported as intact phospholipid molecules to the fetal circulation. The fatty acids liberated, however, may pass the barrier(24). It was suggested as far back as 1905 by Hofbauer that the placenta decomposes large molecular nutrient materials obtained from the mother's blood, and transmits the components to the fetus for further use(25).

Summary. Lipoprotein lipase activity was found to be present in homogenates of placentas of rats pregnant 18-21 days and of human placentas obtained post partum. In the rat, the LPL activity in placenta was equal to that in heart, but much less than that found in adipose tissue. LPL activity in human placenta was smaller than that in rat placenta. Placental lipase activity was inhibited by DFP, protamine and 1 M NaCl, and considerably reduced in the absence of serum in the incubating medium. LPL activity in placenta may be related to the vascularity of this organ and may act to supply the fetus with fatty acids by hydrolyzing lipoproteins obtained from the maternal circulation.

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Effect of Insulin on D-Xylose Uptake by the Intact Rat Diaphragm. (30163)

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The isolated rat diaphragm has been used extensively for bioassay of insulin-like activity. The finding by Kipnis and Cori(1) and Norman *et al*(2), that insulin increased D-xylose penetration into the rat diaphragm has recently been confirmed by Rieser and Rieser(3) using insulin concentrations of 500,000 microunits per ml in *in vitro* incubation systems. These studies suggested that D-xylose penetration might be used to develop a simple and reliable bioassay technique for insulin activity at low insulin concentrations.

The intact diaphragm preparation described by Kipnis and Cori(1) was used since employment of the whole diaphragm eliminates the less specific and less reliable penetration through cut muscle fibers.

Materials and methods. Wistar rats of both sexes weighing between 100 and 150 g were used. The animals were fasted for 16 to 24 hours before being sacrificed by decapitation. The intact diaphragms together with a ring of the ribs were quickly removed, rinsed in Gey and Gey buffer(4) and gently blotted. The diaphragm preparations were placed in beakers in 30 ml of buffer, pH 7.3-7.4, containing 1.8 mg of glucose, 4.5 mg of D-xylose and 2.0 mg of gelatin per ml. Glucose was used in the buffer to preserve better the viability of the membrane and gelatin was added to prevent adsorption of insulin to glass surfaces. Crystalline zinc insulin* was used to prepare test solutions of known concentrations of insulin with the buffer described above. The insulin solutions were added to the diaphragm preparations just prior to incubation in a Dubnoff apparatus at 37°C for 30 minutes under an atmosphere of 95% O_2, 5% CO_2. After incubation, the diaphragms were washed in buffer, blotted and dissected away from the rib cage. The central segment of muscle was weighed and dried to constant weight for determination of water content. The diaphragm tissue was weighed and homogenized in a mortar with sand and water. Proteins were precipitated with Somogyi reagents and a 1-to-20 dilution of the homogenate was prepared. D-xylose content was determined by the method of Roe and Rice (5). The results were expressed as mg of

* Crystalline zinc insulin was kindly suppied by Dr. Otto Behrens, Eli Lilly Co.