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Effects of Pregnancy and Streptozotocin Diabetes on Hepatic Fatty Acid Metabolism (41069)[1,2]

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Abstract. Nonpregnant female rats and pregnant rats were fed *ad libitum*. Diabetes was induced by a single intravenous injection of streptozotocin. Pregnant rats received streptozotocin on Day 17 of gestation. The diabetic rats were used 3 days after treatment with streptozotocin. Protamine zinc insulin and crystalline insulin were administered to diabetic rats subcutaneously 24 hr prior to removal of the livers for perfusion. Livers were removed and perfused *in vitro* in a recirculating liver perfusion apparatus.

Diabetes in nonpregnant and pregnant animals decreased the output of triacylglycerol and increased ketogenesis by the isolated perfused liver in comparison to the corresponding nondiabetic group. In agreement with previous data, pregnancy increased output of triacylglycerol and decreased ketogenesis. Livers from pregnant diabetic rats secreted more triacylglycerol and fewer ketones than did livers from nonpregnant diabetic rats. Pretreatment of diabetic rats with insulin reduced ketogenesis toward normal values and increased output of triacylglycerol to values exceeding corresponding control values. Total net synthesis of triacylglycerol was increased by pregnancy and was decreased in livers from pregnant diabetic rats, when compared to their corresponding controls. The data suggest that certain hormonal changes induced by pregnancy act antagonistically to experimental insulin deficiency.

Maternal serum triacylglycerol concentration is elevated by pregnancy (1) and increased further by diabetes (2). The liver is the primary source of plasma lipids and lipoproteins in the postabsorptive state, and is affected by pregnancy (1) and experimental diabetes (2). However, little information is available on the interactions between diabetes and pregnancy on the metabolism of free fatty acid by the liver. We wished to describe how experimental insulin deficiency affects the metabolism of oleic acid in isolated perfused livers from pregnant rats. The livers were obtained from rats made diabetic by administration of streptozotocin and from diabetic rats treated with insulin. The formation and secretion of triacylglycerol and rates of ketogenesis were used as indices of the effects of diabetes on hepatic fatty acid metabolism in the pregnant rat.

Materials and Methods. Virgin female and male Sprague–Dawley rats were obtained from the Charles River Breeding Laboratories, Wilmington, Massachusetts. Conditions for the maintenance of the animals and mating (1) and perfusion conditions (3) were described elsewhere. Rats were made diabetic by a single intravenous injection of a freshly prepared solution of streptozotocin. Pregnant animals were treated with streptozotocin on Day 17 of gestation. Streptozotocin was dissolved in 5 m*M* citrate buffer, pH 4.6, so that each rat received 70 mg/kg body wt in a volume of 1.0 ml. Diabetic rats were used 3 days after treatment with streptozotocin. Animals were considered to be diabetic if the blood glucose concentration at that time exceeded 20 m*M*. Nonpregnant control rats received 1.0 ml of the citrate buffer without strep-

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tozotocin. Where indicated, diabetic rats received 5 U of crystalline insulin and 5 U of protamine–zinc insulin/100 g body wt, subcutaneously, 24 hr prior to removal of the livers for perfusion.

After a 20-min equilibration period, 5.0 ml of an oleic acid–albumin complex (1), containing 60 μmole of oleic acid, was added as a pulse dose to the perfusate and 136 μmole oleic acid was infused per hour during the 3-hr experimental period; this quantity of oleic acid maintained the average concentration of the free fatty acid in the erythrocyte-free perfusate at 0.28 ± 0.04 m*M* during the experiment.

Nonperfused livers were obtained from normal and diabetic rats that were either nonpregnant or pregnant. The livers were prepared as if for perfusion (1) but homogenized immediately and analyzed. Total lipids in samples of liver homogenate, perfusate, and serum were extracted with chloroform–methanol, 2:1 (v/v) (4); neutral lipids were separated by passage through silicic acid columns (5). Aliquots of the neutral lipid fractions were analyzed for triacylglycerol (6, 7), and free fatty acid (8) by colorimetric methods. Samples of the perfusate containing erythrocytes were hemolyzed and deproteinized with $Ba(OH)_2$–$ZnSO_4$ (9) and aliquots of the protein-free supernatant were analyzed for ketone bodies (10, 11). The statistical significance of the differences between experimental groups was evaluated using a two-tailed table of Student's distribution of *t* (12).

Results. As reported previously, body weight and liver weights increased while the liver-to-body weight ratio was unaffected by gestation (1). In the present study, as previously (1), total uptake of oleic acid by livers in the various experimental groups was identical. Therefore, to assess the metabolic changes occurring in the liver during pregnancy despite the large increases in liver weight, the data for triacylglycerol and ketone body output are reported per liver and as μmole of triacylglycerol or ketone bodies, per μmole of oleic acid taken up (1).

The output of triacylglycerol by livers from nonpregnant, pregnant, and diabetic rats appear in Table I. As reported previously, pregnancy increased output of triacylglycerol by the liver whether the data were expressed per liver or per μmole of free fatty acid taken up by the liver (1). As observed with livers from male animals (13), experimental diabetes in pregnant or nonpregnant females depressed the output of triacylglycerol by the liver. Livers from pregnant diabetic rats, however, secreted more triacylglycerol than did livers from nonpregnant diabetic rats. Livers from diabetic female rats treated with insulin

TABLE I. THE EFFECTS OF PREGNANCY AND DIABETES ON THE OUTPUT OF TRIACYLGLYCEROL BY THE LIVER

	μmole/liver/3 hr	μmole triacylglycerol/ μmole FFA taken up
Group		
A. Nonpregnant (7)	41.24 ± 4.66	0.09 ± 0.01
B. Pregnant (5)	58.92 ± 7.66	0.14 ± 0.01
C. Nonpregnant diabetic (6)	5.70 ± 2.35	0.01 ± 0.01
D. Pregnant diabetic (5)	15.08 ± 1.26	0.04 ± 0.003
Insulin treatment		
E. Nonpregnant diabetic (2)	67.75, 64.65	0.18, 0.18
F. Pregnant diabetic (3)	85.74 ± 3.65	0.22 ± 0.01
Statistical significance		
A vs B	$P < 0.05$	$P < 0.005$
A vs C	$P < 0.001$	$P < 0.001$
B vs D	$P < 0.001$	$P < 0.001$
C vs D	$P < 0.01$	$P < 0.01$

Note. Number of observations are shown in parentheses. Values are means ± SEM.

TABLE II. THE EFFECTS OF PREGNANCY AND DIABETES ON KETOGENESIS BY THE LIVER

	(μmol/liver/3 hr)	(μmol ketone bodies/ μmol FFA taken up)
Group		
A. Nonpregnant (7)	426.2 ± 68.1	1.12 ± 0.19
B. Pregnant (5)	111.3 ± 27.2	0.32 ± 0.08
C. Nonpregnant diabetic (6)	1155.1 ± 102.2	3.01 ± 0.27
D. Pregnant diabetic (5)	750.1 ± 105.8	1.95 ± 0.28
Insulin treatment		
E. Nonpregnant diabetic (2)	53.0, 99.7	0.14, 0.27
F. Pregnant diabetic (3)	93.4 ± 37.3	0.24 ± 0.10
Statistical significance		
A vs B	$P < 0.005$	$P < 0.025$
A vs C	$P < 0.001$	$P < 0.001$
B vs D	$P < 0.005$	$P < 0.005$
C vs D	$P < 0.025$	$P < 0.025$

Note. Number of observations are shown in parentheses. Values are means ± SEM.

actually secreted more triacylglycerol than did the corresponding nondiabetic controls.

The output of ketone bodies by the livers in the various experimental groups appear in Table II. Output of ketone bodies by livers from pregnant rats was decreased compared to livers from nonpregnant rats. Experimental diabetes in nonpregnant and pregnant animals increased ketogenesis by the liver, although the output of ketone bodies by livers from pregnant diabetic rats was less than that by livers from nonpregnant diabetic rats. Pregnant diabetic rats given insulin secreted ketone bodies at the same rate as pregnant nondiabetic controls.

Net formation of triacylglycerol by the livers in the various experimental groups appear in Table III. The net hepatic change of triacylglycerol was calculated as the difference in triacylglycerol concentration between nonperfused and perfused livers. Livers from pregnant animals accumulated more triacylglycerol during the course of perfusion than did livers from nonpregnant rats. The total net change of triacylglycerol (the net hepatic change plus the difference in triacylglycerol secretion) increased in the pregnant compared to nonpregnant rats and decreased in the pregnant diabetic compared with pregnant rats. The variation in the hepatic concentration of triacylglycerol was quite large, particularly in livers from perfused and nonperfused pregnant diabetic rats.

Discussion. Experimental diabetes, induced by streptozotocin in pregnant and nonpregnant animals, favored oxidation rather than esterification of oleic acid by the liver, in contrast to the normal pregnant rat. When the livers were provided with equal quantities of oleic acid, similar observations were made when experimental diabetes was induced in male rats with anti-insulin serum (13) or with alloxan (14). The stimulation of ketogenesis as well as the reduction and secretion of triacylglycerol formation in pregnant diabetics was less profound than in livers from nonpregnant diabetics. Treatment of diabetic animals with insulin reversed the metabolic abnormalities produced by experimental diabetes.

The decreased output of triacylglycerol by livers from diabetic animals, may in part, result from decreased hepatic triacylglycerol synthesis. The release of very-low-density lipoprotein from the liver may be considered as one measure of triacylglycerol synthesis in the liver, since the output of triacylglycerol is proportional to the uptake and esterification of free fatty acid (9). The output of triacylglycerol by livers from fed male rats made diabetic with alloxan (15) or anti-insulin serum (13) is reduced.

TABLE III. THE EFFECTS OF PREGNANCY AND DIABETES ON HEPATIC TRIACYLGLYCEROL FORMATION

	Concentration of hepatic triacylglycerol		Net hepatic change	Output	Total net change
	Perfused (μmole/liver) (A)	Nonperfused (μmole/liver) (B)	(μmole/liver) (A − B)	(μmole/liver) (C)	(μmole/liver) (A − B + C)
Group					
A. Nonpregnant (7)	63.9 ±5.0	76.4 ±14.1	−12.5 ±5.0	41.2 ±4.7	28.7 ±11.5
B. Pregnant (5)	102.3 ±8.1	90.5 ±8.0	11.8 ±8.0	58.9 ±7.7	69.3 ±6.1
C. Nonpregnant diabetic (6)	36.5 ±3.2	38.9 ±0.5	−2.4 ±3.2	5.7 ±2.4	3.3 ±4.9
D. Pregnant diabetic (5)	87.9 ±15.2	74.1 ±10.3	13.9 ±15.2	15.1 ±1.3	28.9 ±15.4
Statistical significance					
A vs B	$P < 0.001$	NS	$P < 0.01$	$P < 0.05$	$P < 0.025$
A vs C	$P < 0.005$	NS	NS	$P < 0.001$	NS
B vs D	NS	NS	NS	$P < 0.001$	$P < 0.05$
C vs D	$P < 0.01$	$P < 0.025$	NS	$P < 0.01$	NS

Note. Number of observations are shown in parentheses. Values are means ± SEM. Output represents amount of triacylglycerol secreted by the livers after 3 hr of perfusion (data of Table I). NS = nonsignificant.

Streptozotocin diabetes was induced in fed female rats 7 days prior to the administration of Triton WR1339 (16). The rate of entry of triacylglycerol into the serum was reduced approximately 50%. Balasse *et al.*, however, reported increased output of triacylglycerol by livers from fed dogs made acutely diabetic by the injection of anti-insulin serum, the output of triacylglycerol was maximal 3–4 hr after administration of anti-insulin serum (17). They also reported that the mean arterial concentration of free fatty acid increased twofold and that the hepatic uptake of free fatty acid approximately doubled. When male rats were treated with anti-insulin serum 5–6 hr prior to perfusion, no differences in triacylglycerol output were observed, when all livers received equal quantities of free fatty acid (18). These data suggest that treatment with anti-insulin serum for 4–5 hr (17) may have been insufficient to produce changes in hepatic lipid metabolism.

Additional evidence for impaired synthesis of triacylglycerol in insulin deficiency is the reduced incorporation of [1-^{14}C]palmitate into triacylglycerol in perfused livers (14) and in homogenates prepared from adipose tissue (19). Further support of the possible role of insulin in regulating triacylglycerol synthesis is the observation that pretreatment of the diabetic groups with insulin 24 hr prior to perfusion of the livers increased the output of triacylglycerol. Finally, support for the role of insulin in the regulation of triacylglycerol synthesis was also obtained by Topping and Mayes (20). They observed enhanced secretion of very-low-density lipoproteins when livers from normal fed male rats were perfused with serum enriched with insulin.

It is of interest that livers from pregnant diabetic rats secreted more triacylglycerol than did livers from nonpregnant diabetic animals. These data suggest that in pregnancy various factors exist which resist the alteration in triacylglycerol metabolism caused by insulin deficiency. Among these factors may be the increased concentrations of plasma estrogens, progesterone (21), and glucocorticosteroids (22) present in pregnancy. It is known that pretreatment of rats with ethinyl estradiol (3) or dexamethasone (23) favors triacylglycerol for-

mation and secretion as well as reduced ketogenesis. The increased secretion of triacylglycerol and the decreased ketogenesis by livers from pregnant diabetic rats compared to livers from nonpregnant diabetic rats, supports the conclusion that hormonal changes induced by pregnancy act antagonistically to conditions resulting from experimental insulin deficiency. In future experiments we shall examine the effects streptozotocin diabetes has on hepatic lipoprotein metabolism.

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