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Increased Maternal-Fetal Transport of Fat in Diabetes Assessed by Polyunsaturated Fatty Acid Content in Fetal Lipids

Robert Goldstein, Emile Levy, Eleazar Shafrir

Shaarei Zedek Hospital, and Department of Biochemistry, Hebrew University, Hadassah Medical School, and Hadassah University Hospital, Jerusalem, Israel

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Abstract. The distribution of fatty acids was determined by gas-liquid chromatography in total lipid and triglyceride fraction of extracts of several tissues of streptozotocin-diabetic rats and their fetuses on day 20 of pregnancy. In maternal rats, diabetes did not significantly affect fatty acid distribution apart from small changes in the relative content of linoleate in adipose tissue and liver. In the placenta, the fetal carcass and the fetal liver the triglyceride content increased approximately 2-fold as a result of maternal diabetes, in association with the elevation in triglycerides and free fatty acids in the maternal circulation.

A pronounced increase in the relative content of linoleate was recorded in the total lipid and triglyceride extracts of placenta (35 and 59%), fetal carcass (56 and 66%) and fetal liver (100 and 205%). Small increases in arachidonate proportion were also seen in some fetal tissues. The large increase in fetal hepatic linoleate indicates that this tissue is an important uptake target of maternal lipids transported in excess into the fetus. The results confirm the previous observations on increased transplacental fat passage in diabetes by demonstrating that the increment in the essential fatty acid, linoleate, parallels the diabetes-induced triglyceride accumulation in the fetoplacental unit.

Introduction

Diabetes in pregnancy is known to produce major changes in metabolite concentration in the maternal circulation. The hyperglycemia was considered quite early to be detrimental to the fetus by promoting increased insulin secretion, excessive tissue growth and obesity [20]. The maternal hyperlipidemia of diabetes, consisting mainly in the elevation of triglyceride (TG)-rich very low-density lipoproteins and free fatty acids (FFA), received attention as a source of fetal lipid much later [2, 16, 19, 23].

Fatty acid transport across the placenta in the nondiabetic condition has been exten-

sively investigated [8, 9, 22] and the passage of FFA and TG across the placenta has been well established.

The extent of transport appears to be species-dependent, ranging from rapid passage in guinea pigs and rabbits [9, 15, 16, 25, 30, 32] to minimal transport in ruminants [13, 18, 31], with intermediate rates of placental crossing in rats [10, 11, 17, 24], primates [21], and women [3, 8, 28].

The possibility that an increased maternal-fetal concentration gradient of TG and FFA in diabetes may promote the passage of lipids across the placenta and contribute to fetal obesity was investigated in human pregnancy [26, 29].

In previous studies in this laboratory a significant correlation between maternal plasma TG or FFA and fetal TG content in the rat [24] was found. Using labelled tracers and following the distribution of maternally-derived fatty acids versus de novo fatty acid synthesis, it was demonstrated that most of the diabetes-induced fetal fat increment originates from preformed maternal TG or FFA rather than from fetal in situ lipogenesis [24].

Linoleic acid is well known as an essential dietary constituent which cannot be elaborated in mammals by fatty acid desaturation. Therefore, the presence of linoleate as well as of other polyunsaturated fatty acids among lipids of fetal tissues necessarily reflects their maternal origin and their content represents a measure of their transplacental passage. The presence of linoleate in rabbit and human fetuses was first observed by *Söderhjelm* [27] who indicated the fetal dependence on the mother as the source of this lipid. Values varying from 25 to 40% of polyunsaturated fatty acid in the TG-fraction of fetal guinea pigs and monkeys have been observed [9]. *Hull and Elphick* [8] estimated, on the basis of human fetal linoleic acid content, that the maternal contribution to the fetal lipid build-up in normal pregnancy may be as high as 50% of total fetal lipid content.

Table I. Levels of maternal and fetal serum glucose, FFA and TG on day 20 of pregnancy in nondiabetic and diabetic rats

Rats	Glucose mmol/l	FFA μmol/l	TG mmol/l
Nondiabetic			
Maternal	5.0 ± 0.2	630 ± 48	3.21 ± 0.25
Fetal	1.6 ± 0.1	165 ± 11	1.33 ± 0.08
Diabetic			
Maternal	22.2 ± 1.5*	1,356 ± 170*	9.45 ± 0.69*
Fetal	14.8 ± 0.4**	271 ± 16**	1.80 ± 0.10*

Values are means ± SE for groups of 18–22 rats. Fetal serum was obtained from pooled blood of each litter. * Difference significant from nondiabetic rats ($p < 0.01$ at least); ** difference significant from fetal nondiabetic rats ($p < 0.01$ at least).

To provide further support for the maternal origin of the fetal fat excess in diabetes, independently of fat quantitation or follow-up of labelled fatty acids, we have compared in the present work the proportion of polyunsaturated fatty acids in the lipids of the feto-placental unit of streptozotocin-diabetic and control rats.

Materials and Methods

Animals and Assays

Female albino rats of the Hebrew University strain, weighing 200–250 g, maintained on a regular stock diet ad libitum, were used. The females were caged with males for a 16-hour period. The 12 h following the mating were counted as half a day of pregnancy. Diabetes was induced on day 13–14 of preg-

Table II. Tissue TG content in maternal rats and their fetuses on day 20 of pregnancy

Rats	Maternal liver	Placenta[a]	Fetal liver[a]	Fetal carcass[a]
Nondiabetic (14)	13.4 ± 1.1	4.07 ± 0.13	4.96 ± 0.24	1.23 ± 0.11
Diabetic (16)	15.6 ± 1.3	9.54 ± 0.42*	11.5 ± 0.50*	2.29 ± 0.18*

Values are means ± SE expressed as μmol/g tissue. Numbers of rats are indicated in parentheses. * Significant difference at $p < 0.01$ at least.

[a] Each value taken for calculation of the means is an average of 3–4 determinations on the placentas and fetuses from the same litter.

nancy by intraperitoneal injection of streptozotocin (55 mg/kg, freshly dissolved in 5 mmol/l citrate buffer, pH 4.5).

On day 20 of pregnancy the animals were decapitated and the maternal blood was collected. Fetal blood was collected into glass capillaries from an incision of the carotid artery and pooled for each litter within 3 min after decapitation. Serum glucose was determined by a hexokinase method, TG by a kinetic, fully enzymatic lipase-glycerokinase spectrophotometric procedure and FFA by a radiochemical assay [7].

The enzymatic determinations were carried out with reagents from Boehringer (Mannheim, FRG) and procedures adapted to the Centrifichem centrifugal analyzer (Union Carbide Rye, N.Y., USA).

The placentas and the fetuses were washed, the maternal and fetal livers were separated and all tissues were extracted in chloroform:methanol [2:1 vol/vol; 5].

Gas-Liquid Chromatography of Fatty Acids

The solvent phases were separated as recommended [5], the clear chlorophorm phase was evaporated and lipids were extracted in a minimum volume of hexane. The lipid components were either directly methylated for analysis of fatty acid distribution in total lipids or first separated on thin-layer chromatography plates (silica gel G) with a solvent system containing hexane/diethyl ether/glacial acetic acid (80:20:3, vol/vol). The different spots were visualized under ultraviolet light after spraying of the plate with Rhodamine 6G (0.01% in absolute ethanol). The TG spot was scraped into small glass tubes and methylated at 60 °C for 16 h by the addition of 1 ml anhydrous methanol containing 2% concentrated sulfuric acid. To each tube 0.5 ml of water was then added, mixed vigorously for 15 s; the layers were allowed to separate and the hexane layer was removed and evaporated to a final volume of 50 μl. Portions were injected into a glass column with 10% SP 2330 on 100/120 chromosorb WAW in a Packard no. 878 gas chromatograph. Standard mixtures of fatty acids (Supelco Inc., no. 4-7040 and 4-7038) were used for the determination of retention times and the calculation of correction factors. The area under the chromatogram peaks was calculated with the aid of Spectra Physics integrator model 603, calibrated with the standard methylated fatty acid mixtures.

Student's t test was used for the evaluation of differences on a nonpaired group basis.

Results

Table I presents the serum metabolite changes on the maternal and fetal side in nondiabetic and diabetic rats. As expected, streptozotocin-diabetes induced a ~4-fold increase in serum glucose, a 2-fold increase in serum FFA and a ~3-fold increase in serum TG in the diabetic mothers. In the fetal serum, apart from a marked 8-fold increase in glucose levels, the elevations in FFA and TG were relatively mild, though significant.

Tissue TG contents are listed in table II. There was only a slight increase in maternal hepatic TG, but a considerable rise (~2-fold)

Table III. Fatty acid distribution in total lipids of maternal and fetal tissues of 20-day pregnant rats

Tissue	Fatty acid						
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
Maternal fat[a]							
Nondiabetic	1.2±0.1	21.9±1.3	2.8±1.3	5.1±0.9	28.7±1.2	36.4±1.3	1.2±0.1
Diabetic	1.3±0.1	21.6±1.4	2.5±0.3	5.8±1.1	32.6±0.8	32.2±1.1*	1.4±0.2
Maternal liver							
Nondiabetic		22.5±0.7	1.4±0.1	24.6±1.6	9.4±0.5	15.8±0.5	23.7±0.7
Diabetic		27.0±0.8	2.0±0.2	21.1±1.5	8.2±0.4	18.9±1.1*	20.1±1.6
Placenta[b]							
Nondiabetic		22.6±1.1	1.6±0.3	20.4±0.6	15.0±0.6	15.8±0.6	19.7±0.6
Diabetic		22.3±1.0	1.4±0.2	16.5±0.4	15.5±0.5	21.4±0.8**	20.0±1.1
Fetal carcass[b]							
Nondiabetic	1.6±0.3	31.7±1.3	3.6±0.4	13.2±0.8	21.5±0.5	7.4±0.7	17.2±1.4
Diabetic	1.4±0.2	29.2±1.5	3.0±0.3	12.7±0.5	19.9±0.6	11.8±0.6**	20.1±0.7
Fetal liver[b]							
Nondiabetic	1.1±0.1	26.9±0.7	3.8±0.2	12.4±0.6	23.9±0.5	8.9±0.8	18.7±0.7
Diabetic	1.0±0.1	23.7±0.3	2.9±0.3	10.5±0.7	21.1±0.4	17.8±1.0**	21.6±1.2*

Values are means ± SE for 8 animals in each group, expressed as percentage of fatty acids recovered from each tissue. Only fatty acids in excess of 1% of total were listed. Those listed accounted for 95.1–98.2% of the total area under the chromatography peaks. * Significant difference between the nondiabetic and diabetic tissue at $p < 0.05$; ** significant difference between the nondiabetic and diabetic tissue at $p < 0.01$.

[a] Samples of perirenal adipose tissue.

[b] For each individual value extracts from randomly pooled 3 placentas, 3 fetuses (from which the liver, stomach and intestines were removed) and 3 fetal livers from the same litter, were used.

in placental, fetal liver and fetal carcass TG concentration.

Fatty acid distribution among the total lipids extracted from the various tissues is recorded in table III. Of the fatty acid distribution characteristic for each tissue, little change could be attributed to diabetes except changes in the relative content of linoleate (C18:2) and, in certain cases, arachidonate (C20:4). In maternal adipose tissue a small but significant decrease in linoleate content was seen together with a small increase in hepatic linoleate content. This was probably a result of diabetes-induced mobilization of stored fat and its transfer to the liver for oxidation. In all tissues of the fetoplacental unit marked increases in the linoleate content were documented in diabetic rats. In the placenta the relative content of linoleate rose by 35% in fetal carcass by 59% and in fetal liver by as much as 100%. The relative content of arachidonate increased only slightly in the placenta and fetal carcass, but more markedly in fetal liver (22%).

Since most of the increment in fetal fat content was confined to the TG fraction,

Table IV. Fatty acid distribution in maternal and fetal tissue triglycerides of 20-day pregnant rats

Tissue	Fatty acid						
	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
Maternal fat[a]							
Nondiabetic	1.6±0.1	26.1±0.9	7.1±0.3	3.6±0.2	31.9±2.1	26.0±0.8	1.0±0.1
Diabetic	1.7±0.1	27.1±1.1	6.8±0.4	3.8±0.2	33.0±1.8	22.3±0.7*	1.2±0.1
Maternal liver							
Nondiabetic	1.1±0.1	38.9±1.1	6.4±0.5	5.5±0.9	29.1±0.9	15.4±0.8	1.9±0.2
Diabetic	1.2±0.1	37.0±1.3	6.0±0.4	5.3±0.7	25.2±1.0	19.5±1.0*	2.5±0.3
Placenta[b]							
Nondiabetic		43.4±1.5	5.4±0.6	22.9±2.0	16.3±2.4	8.1±0.9	1.8±0.4
Diabetic		37.4±1.4	4.8±0.5	23.2±1.9	17.3±1.7	12.6±0.6**	3.4±0.5*
Fetal carcass[b]							
Nondiabetic	2.5±0.3	38.8±1.3	5.0±0.3	13.5±0.5	23.2±0.4	5.8±0.3	8.8±0.6
Diabetic	2.6±0.2	35.9±1.3	4.8±0.3	12.0±0.4	21.9±0.5	9.6±0.5**	9.5±0.6
Fetal liver[b]							
Nondiabetic	2.7±0.6	35.3±1.8	10.6±0.9	15.4±1.7	29.2±2.2	4.3±0.8	1.1±0.4
Diabetic	2.1±0.1	33.6±1.6	9.0±0.6	13.7±0.9	23.1±1.9	13.1±1.3**	2.1±0.5

Only fatty acids in excess of 1% of total were listed. Those listed accounted for 96.5 to 98.6% of the total area under the chromatography peaks. Other values and explanations are the same as for table III.
[a, b] See footnotes a and b in table III.

changes in the polyunsaturated fatty acid distribution were determined in TG isolated from the tissue lipid fractions (table IV). Again, distribution of fatty acids followed a pattern particular for each tissue with little change due to diabetes, apart from linoleate. The relative content of linoleate in the TG fraction of maternal adipose tissue somewhat decreased and in that of the liver increased in diabetic rats in accord with the changes seen previously in total lipids. However, the TG fraction of the tissues of the fetoplacental unit exhibited increases in the relative linoleate content, as the result of diabetes, which were more extensive than those in the total lipid fraction. In the placenta the proportion of linoleate rose by 56%. In this tissue the proportion of arachidonate also rose significantly by 89%, even if this acid constituted, in general, only a small fraction of total fatty acids of tissue TG. In fetal carcass, the relative content of linoleate rose by 66% and the most prominent rise occurred in the liver, amounting to 205%.

Discussion

The remarkable increase in linoleate content in the fetoplacental unit as a result of maternal diabetes confirms and expands the previous findings from our laboratory that the increase in the maternal plasma FFA and TG concentration leads to an increased lipid

transport across the placenta. It is difficult to compare the quantitative rise in fetal fat content with the increased rate of linoleate transfer. It may be noted that as a result of 6–7 days of diabetes duration (from day 13–14 to day 20 of gestation) the TG content of the placenta, the fetal carcass and the fetal liver increased ~2-fold. The increases in the relative proportion of linoleate were < 2-fold in the placenta or carcass total lipids or TG, but > 2-fold in the liver. These increases can be interpreted with certainty as a specific contribution of maternal fat transfer through the placenta, since all linoleate in mammalian species has to be acquired from external sources. Furthermore, the particular increase in the fetal hepatic linoleate content suggests that it is the first and main target of uptake of fatty acids of maternal derivation after their placental deposition and transfer. The hepatic fatty acids are subsequently distributed to other fetal tissues.

To appraise the extent of maternal fat contribution versus intrafetal synthesis, the direct maternal linoleate source should be known, but this may only be assumed. The linoleate content of maternal adipose tissue or liver were 3 or 2 times higher than the content in fetal liver in nondiabetic and diabetic rats, respectively. This implies that no complete equilibration occurred and that fetal de novo fat synthesis importantly contributed other fatty acids. In fact, fetal lipogenesis measured by ^3H_2O incorporation, was not reduced by maternal diabetes [24]. According to theoretical considerations, if linoleate in fetal liver lipids, as the main depot, constituted 9% of fatty acids and maternal adipose tissue (as the main source) contained 36%, it could be assumed that in the non-diabetic rat at least 25% of the fetal fat was derived from the maternal circulation. In the diabetic rat the relative linoleate concentration in the fetal liver rose to 18%, whereas in maternal adipose tissue it decreased to 32%, suggesting that the maternal share of the fetal fat could have risen to 56% at least. Of course, these are rough approximations, as they assume nonselectivity in the FFA transport across the placenta. They also tend to be underestimations, since the linoleate released from the liver to other fetal tissues is disregarded and, in addition, the source of maternal linoleate may be liver as well, which relatively contains less linoleate than adipose tissue. Nevertheless, these approximations are useful to illustrate the magnitude of increase in maternal-fetal fat flow in severe, insulin-deficient rat diabetes (~2-fold) which roughly corresponds to the ~2-fold increase in fetal tissue TG content.

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Prof. E. Shafrir,
Department of Biochemistry,
Hadassah University Hospital,
PO Box 12000,
Jerusalem 91120 (Israel)