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In vivo Glycerol Metabolism in the Pregnant Rat

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Abstract. Pregnant rats at 12 and 21 days of gestation and their virgin controls were injected intravenously with U-^{14}C-glycerol and decapitated 1, 3, or 10 min later. The conversion of labelled glycerol to ^{14}C-glucose was augmented in the 21-day pregnant rats. The disappearance of the newly formed ^{14}C-glucose from blood was faster in both 12- and 21-day pregnant rats than in their controls, being partially retained as liver ^{14}C-glycogen. The greatest amount of radioactivity in all tissues appeared in the carcass hydrosoluble fraction. This amount was smaller in the pregnant rats. The reduced utilization of glycerol by extrahepatic tissues allowed the 21-day pregnant rats to dispose a greater amount of this substrate for gluconeogenesis.

Introduction

The *in vivo* utilization of glycerol for glucose synthesis is very high and proportional to the plasma concentration of the compound (for a recent review, see *Lin*, 23). The precise amount of glucose formed from glycerol is not well established and reported values in the fasted rat range from 75–90% (24) to 18% (5). This discrepancy is partially due to difficulties in determining the endogenous glycerol space (11), because after administration of glycerol, the molecule is metabolized before being homogenized in the plasma. It has recently been observed that the conversion of glycerol to glucose is enhanced in the 21.5-day pregnant rat (11). In a previous study (16), we observed no increase in gluconeogenesis from pyruvate in fed, 21-day pregnant rats. Thus, it was of interest to investigate whether the activity of gluconeogenesis in late pregnancy actually depends on the substrate utilized. In the present study, we have recorded the *in vivo* synthesis of glucose from labelled glycerol in both 12- and 21-day pregnant rats, the former being included in the experimental protocol in an attempt to determine whether the gluconeogenetic activity in the mother changes according to the fetal needs of its major fuel, glucose. Indeed in the 12-day pregnant rat, fetal metabolic draining from the mother is very slight as fetal growth is still low (1). This study has been extended to determine the tissue distribution of radioactivity at short intervals after intravenous administration of the tracer.

Materials and Methods

Wistar rats were mated when weighing 150–200 g. Gestation date was determined by the appearance of spermatozoids in vaginal smears. The pregnant rats were compared with age-mated female virgin controls. All animals were maintained in a temperature (22 ± 2 °C) and light cycle (12 h on-off) controlled environment and were sacrificed by guillotine at 1, 3, or 10 min after the injection of 10 μCi of U-^{14}C-glycerol (32 mCi/mmol) diluted in 0.5 ml saline/rat. Blood was collected from the neck into heparinized beakers and after cold centrifugation, an aliquot of plasma was used for protein precipitation with 10% $HClO_4$. Following neutralization with saturated $KHCO_3$ in the cold, the supernatants were used for the evaluation of glucose (18) and glycerol (9) concentrations, and for the paper chromatographic separation of labelled glucose and glycerol in the upper phase of n-butanol/water/methanol/90.7% formic acid (320/320/80/1, by volume). Samples were chromatographed in the presence of carrier solutions of glucose and glycerol, and parallel aliquots of the injected U-^{14}C-glycerol were always run to make the proper corrections. The paper chromatograms were cut in 1 cm strips to count their radioactivity. A maximum of three peaks were always found corresponding to the Rfs of ^{14}C-lactate, ^{14}C-glucose and ^{14}C-glycerol standards also chromatographed in parallel.

Another aliquot of plasma was used for lipid extraction and purification (7). Immediately after sacrifice, an aliquot of liver was frozen in liquid N_2 for digestion in 30% KOH, glycogen precipitation in 95% ethanol, and quantification of its radioactivity and concentration after acid hydrolysis by analysis of its glucose content (18). The remaining liver and other organs, including the fetus and placenta, were rapidly dissected and placed in ice-cold saline for weighing and lipid extraction and purification (7). 1 m*M* carrier glycerol was used instead of water for the upper phase of the lipid purification and the first three washes of the lipid extracts from each organ or tissue were pooled for determining the radioactivity in the hydrosoluble phase. The same procedure was used for aliquots of the mechanically ground carcass of the animals which included the skeleton and muscles without the skin and viscera. The lipid-soluble fractions were counted in scintillation liquid containing 750 ml xylene, 3 g diphenyloxazol (PPO), 0.1 g 2,2-*p*-diphenyl-bis-15-phenyloxazol (POPOP), and 250 ml triton X-100. The chromatogram strips, glycogen hydrolysates, and hydrosoluble fractions obtained after the lipid extractions were counted in scintillation liquid containing 1 g PPO, 0.01 g POPOP, 16 g naphthalene, and 200 ml of xylene/dioxane/95% ethanol (5/5/3, by volume). The radioactivity was counted in a Nuclear Chicago scintillator (Isocap-300), provided with external standard for continuous quenching correction.

Plasma volume was calculated by determining the isotopic dilution of ^{131}I-labelled albumin (26) in 12- and 21-day pregnant rats and their controls 10 min after its intravenous injection, giving values of 14.2 ± 1.0, 18.4 ± 0.9, and 11.7 ± 1.1, respectively. Glucose space was considered to be 30% of body weight for all animals (8) and on the basis that pregnancy does not alter this parameter (12, 27). The statistical comparison among the groups was performed with the Student's t test (6) by using 445- and 326-Compucorp electronic calculators.

Results

As shown in table I, the plasma concentration of glycerol was unchanged in 12-day and increased in 21-day pregnant rats as compared to controls. In contrast, plasma glucose levels decreased in the 21-day pregnant rats and were not significantly altered in the 12-day subjects (table I). The disappearance of U-^{14}C-glycerol from plasma is very rapid because 1 min after its intravenous injection, no more than 7% of the injected radioactive substance remains in the total plasma (table II). In 21-day pregnant animals, the ^{14}C-glycerol disappearance rate is slower than in the other groups, its amount remaining in plasma at 1 and 10 min, being significantly higher than in controls, with no differences between the 12-day pregnant rats and controls or in the plasma ^{14}C-glycerol specific activity in the three groups (table II).

The production of glucose from glycerol is very rapid and 1 min after injection of the

tracer, 20–30% of the radioactivity is already present in the glucose space in the form of glucose (table II). The conversion of ^{14}C-glycerol to glucose is greater in 21-day pregnant rats than in the controls at 1 and 3 min, while at 10 min the values of labelled glucose were significantly reduced (table II). Plasma glucose-specific activity was similar in the two groups of pregnant rats and the controls 1 and 3 min after injection of ^{14}C-glycerol but was significantly reduced in comparison to controls at the 10-min period. To check whether these differences in the ^{14}C-glucose values were influenced by changes in its conversion to glycogen, the appearance of radioactivity in whole liver glycogen was also determined (table II). At all the

Table I. Effect of pregnancy on plasma glycerol and glucose levels in the rat

	Gestational age		
	0 days	12 days	21 days
Plasma glycerol, μmol/l	63.77 ± 7.14	66.32 ± 7.65	91.83 ± 7.93*
Plasma glucose, mg/100 ml	111.60 ± 12.31	95.42 ± 10.21	86.01 ± 6.21**

The values correspond to means ± SEM of 14–15 animals per group. Asterisks indicate the statistical comparison between each group of pregnant rats and the virgin controls (0 days of gestation): * $p < 0.05$; ** $p < 0.01$; no asterisk denotes $p > 0.05$.

Table II. Effects of pregnancy of the appearance of ^{14}C-glucose in the circulation and hepatic glycogen in the rat after the intravenous injection of 10 μCi of U-^{14}C-glycerol/animal

Parameter	Gestational Age		
	0 days		
	1 min[1]	3 min	10 min
^{14}C-Glycerol in plasma,			
% of injected radioactivity in whole plasma	4.11 ± 0.52	1.80 ± 0.16	0.81 ± 0.13
Plasma glycerol SA, dpm/μmol	60,508 ± 5,080	14,732 ± 2,286	7,112 ± 1,524
^{14}C-Glucose formed,			
% of injected radioactivity in glucose space	26.2 ± 2.5	29.6 ± 2.7	24.3 ± 3.2
Glucose SA, dpm/μmol	738 ± 81	859 ± 108	936 ± 125
Liver ^{14}C-Glycogen,			
% of injected radioactivity in whole liver	0.021 ± 0.004	0.142 ± 0.009	0.023 ± 0.003
Liver glycogen SA, dpm/μmol of glucose	1.02 ± 0.15	4.24 ± 0.48	1.12 ± 0.15

[1] Minutes after glycerol administration.

times samples were taken after injection of the tracer, both total ^{14}C-glycogen and ^{14}C-glycogen specific activity were significantly higher in the 21-day pregnant rats compared with their controls, with no differences between the 12-day pregnant rats and their controls at 1 and 3 min, while these activities increased in the former group at 10 min.

The slower disappearance of ^{14}C-glycerol from plasma and its greater conversion to glucose in the 21-day pregnant rat should be the result of a decreased utilization of that substrate by other tissues. The values of radioactivity in different organs and tissues distributed in hydro- and liposoluble fractions are shown in table III. When compared to the values in the controls, the radioactivity of both fractions in liver was augmented in the 21-day pregnant rats at 1 and 3 min; in parametrial adipose tissue, the radioactivity of the hydrosoluble fraction was high at 3 min in the 12-day pregnant rat, while in the liposoluble fraction, it was high at 1 min in the 21-day pregnant rat and at 10 min in the 12-day animals; also, it was reduced at 3 and 10 min in the 21-day pregnant animals. In the kidneys, both the hydro- and liposoluble fractions had lower radioactivity in the two groups of pregnant rats at 1 min after the injection of the tracer, while in the heart, this is the case for the hydrosoluble fraction at 1 min and the liposoluble fraction at 10 min in the 21-day pregnant animals.

From all the organs and tissues, the carcass incorporates the highest percentage of radioactivity, mainly in the hydrosoluble fraction which is actually reduced in the 21-day pregnant rats at 1 and 3 min and in the 12-day pregnant rats at 3 min, while the lipidic fraction is augmented in the former group at 1 min. The radioactivity in the fetus of 12 days is minimum, while in the fetus of 21 days it is significant, most of it being present in the hydrosoluble form which increases as a function of time after the injection of the tracer to

12 days			21 days		
1 min	3 min	10 min	1 min	3 min	10 min
4.01 ± 0.55	1.67 ± 0.25	0.55 ± 0.06	6.95 ± 0.64**	2.75 ± 0.22	2.27 ± 0.27**
48,958 ± 5,588	11,684 ± 2,032	4,826 ± 1,016	50,228 ± 4,953	19,558 ± 2,667	7,620 ± 2,032
19.5 ± 2.2	30.1 ± 3.6	14.4 ± 1.9*	31.3 ± 3.6	40.5 ± 2.5**	24.3 ± 2.1
540 ± 79	769 ± 117	589 ± 58*	809 ± 139	895 ± 72	535 ± 42*
0.030 ± 0.006	0.131 ± 0.014	0.051 ± 0.004***	0.081 ± 0.008***	0.203* ± 0.019*	0.122 ± 0.018***
0.97 ± 0.17	3.67 ± 0.25	2.01 ± 0.28*	2.73 ± 0.25**	6.38 ± 0.72*	3.05 ± 0.17***

The values correspond to means ± SEM of 4–6 animals per group. Asterisks indicate the statistical comparison between each group of pregnant rats and the virgin controls (0 days of gestation): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; no asterisk denotes $p > 0.05$.

the mother. A minimum proportion of radioactivity is found in the hydrosoluble fraction of the placenta of 21 days, being negligible in the liposoluble fraction.

Discussion

The rapid disappearance of intravenously injected glycerol from plasma observed in the present study confirms the results of others (11) and does not seem due only to the dilution of the tracer into a great volume (17), but to its rapid uptake and metabolization by different organs and tissues. Our data indicate that the carcass, followed by the liver, are the two tissues with the highest percentage of glycerol utilization. 1 min after injection, they had almost 40% of radioactivity. During this first minute, the greatest amount of radioactivity appeared in the hydrosoluble fraction of all tissues studied, after which there was a decline in most of them, with an increase of radioactivity of the lipidic fraction. This was specially

Table III. Effects of pregnancy on tissue appearance of radioactivity as hydro- (H) or liposoluble (L) fraction in the rat after the intravenous injection of 10 μCi of U-^{14}C-glycerol/animal

Organ or tissue	Fraction	Gestational age		
		0 days		
		1 min[1]	3 min	10 min
Liver	H	8.06 ± 0.96[2]	4.01 ± 0.85	3.01 ± 0.52
	L	0.92 ± 0.14	1.25 ± 0.19	2.60 ± 0.51
Kidney	H	1.00 ± 0.11	0.42 ± 0.06	0.27 ± 0.05
	L	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.03
Parametrial adipose tissue	H	0.027 ± 0.005	0.018 ± 0.001	0.011 ± 0.003
	L	0.005 ± 0.001	0.020 ± 0.001	0.022 ± 0.003
Heart	H	0.43 ± 0.04	0.26 ± 0.03	0.22 ± 0.02
	L	0.014 ± 0.001	0.021 ± 0.002	0.027 ± 0.004
Carcass	H	25.01 ± 2.20	29.57 ± 1.60	23.01 ± 2.97
	L	0.58 ± 0.05	1.43 ± 0.19	1.60 ± 0.16
Fetus	H			
	L			
Placenta	H			
	L			

[1] Minutes after injection.
[2] % of injected radioactivity in whole organ or tissue.

true in the liver and adipose tissue where the use of glycerol as a lipogenetic substrate may be prominent (4, 14, 15). Part of the radioactivity used for lipid synthesis obviously may come from the newly formed, labelled glucose. Its synthesis from glycerol is extremely rapid as observed in our work and as shown by other authors (11).

Pregnancy produces important changes in these relationships. The slower disappearance of ^{14}C-glycerol from plasma in the 21-day pregnant rat did not signify a reduced utilization but was a consequence of its greater dilution by the higher circulating glycerol levels. Actually, the specific activity of the plasma glycerol was unchanged, comparing pregnant animals with controls. The use of labelled glycerol was considerably increased in the liver of the 21-day pregnant animal and this was partly due to a concomitant augmented lipogenesis and gluconeogenesis. The latter was well documented by the enhanced appearance of labelled glucose in the circulation and also by the greater incorporation of radioactivity in liver glycogen. Both increases were in terms of absolute DPM and of specific activity. This augmented gluconeogene-

12 days			21 days		
1 min	3 min	10 min	1 min	3 min	10 min
8.13 ± 1.22	6.44 ± 0.88	2.75 ± 0.53	10.11 ± 0.52**	8.75 ± 0.73***	4.22 ± 0.22
0.61 ± 0.09	2.05 ± 0.40	1.52 ± 0.23	1.88 ± 0.34*	2.37 ± 0.41*	3.88 ± 0.61
0.64 ± 0.04***	0.47 ± 0.07	0.25 ± 0.03	0.51 ± 0.08***	0.39 ± 0.02	0.29 ± 0.03
0.06 ± 0.01***	0.13 ± 0.03	0.17 ± 0.02	0.09 ± 0.01*	0.10 ± 0.01	0.09 ± 0.02
0.031 ± 0.002	0.063 ± 0.003***	0.020 ± 0.002	0.021 ± 0.003	0.023 ± 0.002	0.014 ± 0.002
0.006 ± 0.001	0.023 ± 0.002	0.034 ± 0.001***	0.009 ± 0.001**	0.010 ± 0.001***	0.008 ± 0.001***
0.41 ± 0.028	0.31 ± 0.018	0.18 ± 0.032	0.31 ± 0.03*	0.29 ± 0.02	0.26 ± 0.02
0.0 ± 0.002	0.020 ± 0.002	0.021 ± 0.002	0.013 ± 0.002	0.018 ± 0.002	0.009 ± 0.001**
21.32 ± 2.85	19.25 ± 2.03*	15.44 ± 1.85	17.91 ± 1.82*	19.45 ± 0.87*	15.01 ± 1.74
0.59 ± 0.06	1.02 ± 0.23	1.58 ± 0.21	1.28 ± 0.23	1.33 ± 0.19	1.40 ± 0.29
0.38 ± 0.09	0.52 ± 0.10	0.50 ± 0.20	1.09 ± 0.21	2.61 ± 0.78	4.74 ± 0.78
0.049 ± 0.006	0.112 ± 0.010	0.097 ± 0.029	0.048 ± 0.020	0.078 ± 0.018	0.128 ± 0.031
–	–	–	0.58 ± 0.08	0.59 ± 0.05	0.45 ± 0.07
–	–	–	0.010 ± 0.001	0.024 ± 0.005	0.019 ± 0.002

The values correspond to means ± SEM of 4–6 animals per group. Asterisks indicate the statistical comparison between each group and the virgin controls (0 days of gestation): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; no asterisk denotes $p > 0.05$.

sis from glycerol in the fed mother rat was not found when ^{14}C-pyruvate was used as a substrate (16).

This finding may be related to a preferential use of glycerol released through lipolysis which is increased near term (19, 20). The mother has in this way an alternative source of glucose to compensate for its continuous draining by the fetus. From 3 to 10 min after its injection, when the labelled glycerol remaining in plasma was very low, the decrease of plasma glucose specific activity in the mother corresponded with the increased appearance of hydrosoluble radioactivity in the fetus. This finding suggested that besides the rapid transfer of some injected ^{14}C-glycerol across the placenta (10), the newly formed glucose was the main product of glycerol metabolism utilized by the fetus.

It is intersting that in the pregnant rat the augmented conversion of glycerol to glucose occurred at the expense of reduced utilization of this substrate by other tissues of the mother, including kidneys, heart, and carcass, the latter being quantitatively the most important. A possible interpretation of this finding was that the augmented resistance to insulin in late pregnancy (3, 21) and the preferential use of lipidic products by the mother's structures (25) contributed to the preservation of glycerol for its use by the fetus either directly or after its previous transformation to glucose. Although its quantitative contribution was very small, the present study indicates that 1 min after injection of the tracer, there was a greater percentage of radioactivity in the liposoluble fraction of the 21-day mother's adipose tissue. These results agree with our recent observation about the *in vitro* enhanced utilization of glycerol by the adipose tissue from pregnant rats (2) and would suggest the direct use of glycerol by this tissue, contributing to the net deposition of fat stores in the mother.

The metabolism of glycerol in the 12-day pregnant rat is less affected than in the 21-day animal, probably due to the smaller fetal size which causes less drainage of metabolic fuels from the mother. At this time of pregnancy, the lipidic metabolism of the mother is still almost normal (22, 25) and the physiological levels of plasma glycerol in the mother would suggest an unchanged, endogenous lipolysis. In any case, our data indicate that in 12-day pregnant animals, there was already a change in the rapid use of glycerol in certain organs (kidney and carcass), an augmented disappearance of the newly formed glucose from the mother, and an augmented accumulation of radioactivity in liver glycogen and in adipose tissue lipids. All these findings suggest that the 12-day gestating mother starts accumulating energetic stores in preparation for the onset of rapid growth of the fetus (1). A similar explanation has been proposed for the lipoprotein lipase activity in adipose tissue of the mother (13, 22), providing an explanation for her anabolic condition in spite of the continuous metabolic draining by the fetus.

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