Role of the Availability of Substrates on Hepatic and Renal Gluconeogenesis in the Fasted Late Pregnant Rat

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Studies were conducted to examine the role of gluconeogenetic substrate availability on glucose production in the fasted late pregnant rat. Virgin and 21-day pregnant rats were studied after 24 hours' food deprivation. Pregnant animals showed decreased circulating glucose and gluconeogenic amino acid and increased plasma glycerol concentration. Glucose formation was studied in vivo two, five, and ten minutes after the intravenous administration of two concentrations of ^{14}C-alanine, ^{14}C-pyruvate, or ^{14}C-glycerol. Concentrations of 0.2 mmols of ^{14}C-glycerol or ^{14}C-pyruvate, but not of ^{14}C-alanine, enhanced ^{14}C-glucose production in pregnant rats, whereas 1 mmol of any of the three ^{14}C-substrates always enhanced ^{14}C-glucose production in these rats. Both 1 mmol/L and 5 mmol/L ^{14}C-alanine increased ^{14}C-glucose formation in 90-minute–incubated liver slices of fasted pregnant rats, in spite of decreased cytosolic activity of alanine aminotransferase. The three substrates enhanced "in vitro" renal gluconeogenesis in pregnant rats. Under all experimental conditions studied, labeled glycerol was converted more efficiently into glucose than equivalent amounts of any other substrate used, and this difference was greater in pregnant, than in virgin animals. Results indicate that, in spite of enhanced gluconeogenetic activity, maternal glucose production in the fasted state at late gestation is limited by the deficiency of certain substrates, such as amino acids. It is proposed that glycerol derived from enhanced maternal adipose tissue lipolysis constitutes a preferential gluconeogenetic substrate in comparison with others, such as alanine, that are more efficiently transferred through the placenta to the fetus.

FASTING during late pregnancy is known to cause marked hypoglycemia.[1] Since fetal metabolism is highly dependent on glucose for oxidative purposes,[2] it has been suggested that increased fetal glucose utilization may cause maternal hypoglycemia.[3] It has been reported, however, that during fasting at late pregnancy, hypoglycemia precedes increased glucose turnover,[4] indicating impairment of glucose synthesis.

Glucose production during fasting occurs by gluconeogenesis mainly in liver, but also in kidney cortex. In the fasted pregnant rat, the rate of in vivo gluconeogenesis is greatly increased from pyruvate.[1] Concerning the availability of gluconeogenic substrates, during fasting at late pregnancy, plasmatic glycerol levels rise due to accelerated triglyceride breakdown in adipose tissue,[5-7] but hypoaminoacidemia exists.[8-10]

Due to the differential effect of anesthetics on in vivo gluconeogenesis previously reported in 24-hour–starved virgin and pregnant rats,[11] in the present study we examined the significance of the availability of different gluconeogenic substrates in unanesthetized fasted rats at late pregnancy to determine whether, in this special metabolic situation, gluconeogenic pathways in liver and kidney cortex are modified to alleviate hypoglycemia. The glucose production was studied in in vivo and in vitro incubations of liver and kidney cortex slices from rats treated with different amounts of alanine, pyruvate, and glycerol.

MATERIALS AND METHODS

Animals

Sprague-Dawley female rats from our own colony weighing 160 g were mated, and gestation was timed from the appearance of spermatozoids in vaginal smears. Sex-matched and age-matched virgin rats were studied in parallel. Animals were housed in collective cages in a light cycle controlled and temperature controlled room (12 hours on–off; 23 ± 1 °C) and fed ad libitum with Purina chow pellets (Panlab, Barcelona, Spain). In all studies, 24-hour–fasted, 21-day pregnant rats and their virgin controls were used.

Analysis of Steady-State of Metabolites and Enzyme Activities

Blood samples were collected from the neck after decapitation of the unanesthetized animals. Protein precipitation was performed in plasma aliquots with $ZnSO_4$ and Ba $(OH)_2$[12] for glucose analysis,[13] whereas 6% $HClO_4$ was used for the analysis of lactate,[14] pyruvate,[15] and glycerol,[16] and 10% sulfosalycilic acid in 0.1 N HCl was used for amino acid analysis with a Beckman 121 MB autoanalyzer (Beckman Co, Palo Alto, Calif). Immdediately after killing the animals, an aliquot of liver was placed in liquid nitrogen for glycogen purification[17] and determination.[1] Another aliquot of liver and a portion of dissected kidney cortex, processed fresh the day of death, were homogenized in 0.25 mol/L sucrose containing 0.001 mol/L EDTA-Na_2, pH 7.0, and after centrifugation at 1000 g for 15 minutes, the supernatant was centrifuged at 16,300 g for 2 minutes. The supernatant of this second centrifugation was directly used as a cytosolic fraction, whereas the pellet was redissolved and centrifugated again at 16,300 g for 20 minutes with the same medium, and the resulting pellet was used as a mitochondrial fraction. Another aliquot of fresh liver was directly homogenized in 200 mmol/L phosphate buffer, pH 7.4. Aliquots of both subcellular fractions and

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the total liver homogenate were assayed for alanine aminotransferase[18] and aspartate aminotransferase activities.[19]

Glucose Distribution Space

The method of Baker et al[20] was used to determine glucose distribution space in 24-hour–fasted pregnant and virgin rats. Nembutal-anesthetized animals were injected with 5 μCi of glucose ^{14}C (ul) per 200 g body weight (The Radiochemical Centre, Amersham, Bucks, U.K.) through a femoral vein. At 1, 2, 4, 6, 8, 10, 20, and 30 minutes after tracer administration, blood samples were taken from a cannula placed in the left carotid artery. Deproteinized[12] blood or plasma was used to purify ^{14}C-glucose by ion-exchange chromatography.[1] The glucose distribution space (GDS) was calculated as previously described.[21]

In Vivo Experiments

Unanesthetized pregnant and virgin rats were used. In one group of experiments 10 μCi of alanine ^{14}C (ul), pyruvate ^{14}C (3), or glycerol ^{14}C (ul) (The Radiochemical Centre, Amersham, Bucks, U.K.) containing 0.2 mmol per 200 g body weight were injected through a tail vein. In another group, each animal was injected with 1 mmol of one of the three radioactive substrates. Blood samples were collected from the tip of the tail two and five minutes after administration of the tracer. Animals were decapitated at 10 minutes after tracer administration, and blood was collected from the neck wound into heparinized tubes. Blood aliquots were deproteinized[12] with $Ba(OH)_2 - ZnSO_4$ and supernatants were used for glucose determination and for ^{14}C-glucose purification. In experiments using alanine ^{14}C (ul) and pyruvate ^{14}C (3), ^{14}C-glucose purification was done by ionic exchange column chromatography.[1] With this technique, recovery of glucose ^{14}C (ul) added to blood before protein precipitation was over 98.7%, whereas less than 0.19%, 0.22%, and 0.22%, of alanine ^{14}C (ul), pyruvate ^{14}C (3), and lactate ^{14}C (ul), respectively, were recovered in the same fraction, when also added to blood. When alanine ^{14}C (ul) was the injected tracer, another aliquot of blood, different from the one used for glucose purification, was used to obtain plasma that was deproteinized with 10% sulfosalycylic acid in 0.1 N HCl. The supernatants were used for alanine quantification with a Beckman 121 BM amino acid analyzer and for purification of ^{14}C-alanine throughout cation microcolumns (2.5 cm × 4 mm) (AG 50 W × 8 from Bio Rad Laboratories, Richmond, Va) eluted with 3 mL of distilled water and 3 mL of 2 N ammonia. With this technique, recovery of alanine ^{14}C (ul) added to the plasma was over 92.5%, whereas that of glucose ^{14}C (ul) was less than 0.37%.

When glycerol ^{14}C (ul) was used as tracer, ^{14}C-glucose and ^{14}C-glycerol were separated from plasma using ascending paper chromatography.[22] With this technique, recovery of glucose ^{14}C (ul) and glycerol ^{14}C (ul) was 92.3% and 85.5%, respectively. Plasmatic concentrations of glycerol were determined as indicated above

When injecting 0.2 mmol per 200 g body weight of tracer, ^{14}C-glucose formation was expressed as dpm of the ^{14}C-glucose found in the glucose distribution space, corresponding to 200 g of body weight. When 1 mmol was injected, results were expressed in dpm of ^{14}C-glucose in the total glucose distribution space of the animal. All radioactivity values were adjusted by considering 1×10^6 dpm to be the administered tracer.

In Vitro Incubation of Liver and Kidney Cortex Slices

Rats were decapitated, and liver and kidneys were immediately placed in ice-cold 0.9% NaCl. Slices of liver and kidney cortex were cut 0.5 mm wide with a tissue chopper (McIlwaine, U.K.) and placed in Krebs-Ringer bicarbonate buffer pH 7.4.[23] The slices were weighed and placed in counting vials (100 to 120 mg in each vial) containing 1 mL of cold Krebs-Ringer bicarbonate buffer, and 1 μCi of either alanine ^{14}C (ul), pyruvate ^{14}C (3), or glycerol ^{14}C (ul). The media were always supplemented with either 1 or 5 μmol/mL of the respective cold substrate. Zero-time vials, to which 0.1 mL of 1 N H_2SO_4 was added simultaneously with the tracer, as well as blank vials without tissue slices, were used in each experiment. Incubations were performed for 90 minutes and (^{14}C)-CO_2 was collected in hydroxide of hyamine after adding 0.1 mL of 1 N H_2SO_4 to the media, as previously described.[24,25] After centrifugation of the media, aliquots of supernatants were used for purification of different fractions of the radioactivity. When alanine ^{14}C (ul) was studied, samples were placed on microcolumns (4 mm id) filled with AG 50 W × 8 resin in its H^+ form (Bio Rad Laboratories, Richmond, Va) up to 2.5 cm level of the column and up to 5 cm with AG 1 × 8 resin in its OH^- form (Bio Rad Laboratories, Richmond, Va). Microcolumns were eluted with 3 mL of distilled water for the collection of ^{14}C-glucose, followed by the elution with 3 mL of 0.5 N formic acid for the collection of ^{14}C-lactate, and with 3 mL of 2 N ammonia for the collection of ^{14}C-alanine. With this procedure, mean recoveries of glucose ^{14}C (ul), lactate ^{14}C (ul), and alanine ^{14}C (ul) added to the nonradioactive incubation media were 96.6%, 88.0%, and 90.1% in their respective fractions, whereas contaminations in other fractions never exceeded 0.4%. When the labeled substrate was pyruvate ^{14}C (3), samples were added to microcolumns filled with AG 1 × 8 in formate form on the bottom (Bio Rad Laboratories, Richmond, Va) and AG 50 W × 8 in its H^+ form on the top. Elutions were done with 3 mL of distilled water for the collection of ^{14}C-glucose, with 3 mL of 0.5 N formic acid for the collection of ^{14}C-lactate and 5 mL of 3 N formic acid for collection of pyruvate. Recoveries for glucose ^{14}C (ul), lactate ^{14}C (ul), and pyruvate ^{14}C (3) were 97.0%, 91.4%, and 70.3%, respectively. Separation of metabolites derived from glycerol was performed by means of ascending paper chromatography.[22] Recoveries for glucose ^{14}C (ul), lactate ^{14}C (ul), and glycerol ^{14}C (ul) were 92.4%, 90.3% and 85.8%, respectively. Data of glucose formation and substrate uptake by tissue were calculated as the μmols of substrate that were converted into glucose or had disappeared from the medium after one hour of incubation per g of wet tissue. Statistical comparisons between groups were always done with the Student's *t* test.

RESULTS

In Vivo Experiments

In Table 1 are shown the main corporal and metabolic parameters in 24-hour–fasted 21-day pregnant and virgin rats. Body weight was 50% higher in pregnant rats than in their virgin controls. This increase is known to be due not only to the presence of the conceptus but also to the increase in maternal tissues. Regarding the gluconeogenic organs, liver weight increased significantly in pregnant rats, whereas kidney weight did not differ in the two groups (Table 1).

Hypoglycemia during fasting was marked during late gestation (Table 1), while there were no significant changes in glucose distribution space (calculated per g of body weight) or liver glycogen concentration. Concerning the plasmatic levels of gluconeogenic substrates, lactate and pyruvate remained stable, gluconeogenic amino acids decreased significantly, and glycerol increased in pregnant rats *v* controls (Table 1), in agreement with earlier studies.[1,8]

In order to study in vivo glucose production, 0.2 mmol per 200 g body weight of ^{14}C-labeled alanine, pyruvate, or glycerol were intravenously injected in unanesthetized, 24-

Table 1. Corporal and Metabolic Parameters in 24-hour–Fasted 21-Day Pregnant Rats

	Virgin	21-day Pregnant
Body weight (g)	231 ± 9	345 ± 13‡
Liver weight (g)	6.0 ± 0.2	9.5 ± 0.3‡
Kidneys weight (g)	1.3 ± 0.1	1.4 ± 0.1
Blood glucose (mmol/L)	4.2 ± 0.3	3.0 ± 0.3§
*Glucose distribution space (ml/100 g body weight)	28 ± 1	34 ± 4
Hepatic glycogen (%)	0.008 ± 0.002	0.010 ± 0.006
Plasma lactate (mmol/L)	5.2 ± 0.6	4.7 ± 0.6
Plasma pyruvate (umol/L)	181 ± 33	158 ± 37
†Plasma gluconeogenic amino acids (mmol/L)	2367 ± 167	1869 ± 86§
Plasma glycerol (μmol/L)	143 ± 11	245 ± 17‖

Values are given as the Mean ± SEM of 6–10 rats per group.

*Glucose distribution space was determined in different animals than the other parameters studied under nembutal anaesthesia.

†Plasma gluconeogenic amino acids correspond to the sum of Ala, Glu, Gln, Asp, Asn, Ser, Gly, and Thr.

Statistical comparisons between values from pregnant and virgin rats: ‡$P < 0.001$, §$P < 0.05$, ‖$P < 0.01$.

hour–fasted 21-day pregnant and virgin rats, and results are presented in Table 2. ^{14}C-glucose production increased significantly in pregnant rats two, five, and ten minutes after administration of pyruvate, and 5 minutes after administration of glycerol. When alanine was the tested gluconeogenic substrate, however, no change in ^{14}C-glucose was observed. As shown in Table 3, at 10 minutes after tracer injection in the corresponding experiments, plasma values of ^{14}C-glycerol and ^{14}C-alanine did not differ in pregnant and virgin animals. Plasma concentration of glycerol remained stable in both groups of animals, while alanine was significantly reduced in pregnant rats (Table 3). Specific activities of circulating ^{14}C-glycerol or ^{14}C-alanine did not differ between pregnant and virgin rats (Table 3).

Since the action of alanine as a gluconeogenetic substrate in pregnant rats differed from the actions of glycerol and pyruvate, hepatic and renal cortex activities of alanine and

Table 2. ^{14}C-Glucose Formation in Fasted 21-day Pregnant Rats, After 0.2 mmol of Alanine ^{14}C (ul), Pyruvate ^{14}C (3), or Glycerol ^{14}C (ul)

Minutes After the Tracer	dpm × 10^{-2} of ^{14}C-Glucose/200 g Body Weight		
	2	5	10
Pyruvate ^{14}C (3)			
Virgin	361 ± 22	662 ± 53	1724 ± 72
Pregnant	546 ± 82*	1023 ± 111*	1979 ± 81*
Glycerol ^{14}C (ul)			
Virgin	1048 ± 190	1409 ± 127	3250 ± 222
Pregnant	1312 ± 117	1794 ± 62*	3071 ± 263
Alanine ^{14}C (ul)			
Virgin	274 ± 18	414 ± 67	1036 ± 171
Pregnant	282 ± 30	483 ± 58	1203 ± 166

Values are given as the Mean ± SEM of 6 to 10 rats/group.

Unanaesthetized rats were IV injected with the tracer (10 μCi/0.2 mmol/200 g body weight).

*Statistical comparisons between values from pregnant and virgin rats ($P < 0.05$).

Table 3. Plasma-Specific Activities of Alanine ^{14}C (ul) and Glycerol ^{14}C (ul) After 10 Minutes of 0.2 mmol Administration in 21-Day Pregnant Rats

	^{14}C-Substrate (dpm/mL)	Plasma Substrate Concentration (μmol/L)	^{14}C-Specific Activity (dpm/μmol)
Glycerol ^{14}C (ul)			
Virgin	1398 ± 99	577 ± 51	2558 ± 143
Pregnant	1806 ± 184	648 ± 29	2923 ± 245
Alanine ^{14}C (ul)			
Virgin	1471 ± 97	1150 ± 51	1283 ± 77
Pregnant	1301 ± 62	819 ± 68*	1486 ± 89

Values are given as Mean ± SEM of 6 to 10 rats/group.

Unanaesthetized rats were IV injected with the tracer (10 μCi/0.2 mmol/200 g body weight).

*Statistical comparisons between pregnant and virgin animals ($P < 0.01$).

aspartate aminotransferases were assayed. These results, presented in Table 4, indicated that in pregnant rats, total and cytosolic activities of alanine aminotransferase were decreased in liver, while there was no change in mitochondrial isoenzyme activity. No significant differences between pregnant and virgin groups were detected for this enzyme in the kidney cortex, where activity values of both the cytosolic and mitochondrial isoenzymes were lower than in the liver. Total hepatic aspartate aminotransferase activity was significantly increased in pregnant *v* virgin rats, but this difference was not detected when assayed in either cytosolic or mitochondrial fractions. No differences were found in renal activities of aspartate aminotransferase in the two groups (Table 4).

In view of the unaffected rate of gluconeogenesis following injection of 0.2 mmol of alanine per 200 g body weight and the reduced activity of liver alanine aminotransferase in pregnant rats, another series of experiments was performed administering intravenously 1 mmol of labeled substrate per rat and determining ^{14}C-glucose production. As shown in Table 5, this parameter was significantly higher in pregnant than in virgin rats five minutes after glycerol ^{14}C (ul) and two, five, and ten minutes after either pyruvate ^{14}C (3) or alanine ^{14}C (ul). Since the same amount of labeled substrate was injected to both pregnant and virgin rats, the decreases in ^{14}C-alanine and ^{14}C-glycerol values and in their chemical concentrations found in plasma of pregnant rats (Table 6) were evidently due to their greater body weight and consequently augmented endogenous dilution of the administered tracer. Furthermore, as shown in Table 6, plasma ^{14}C-glycerol and ^{14}C-alanine values, as well as plasma concentration of glycerol and alanine, in these animals injected with 1 mmol of the respective substrate were higher than in those receiving 0.2 mmol per 200 g body weight (Table 3). Plasma specific activity of either ^{14}C-glycerol or ^{14}C-alanine did not differ between pregnant and virgin rats receiving 1 mmol of labeled substrate (Table 6), as it also occurred with 0.2 mmol per 200 g body weight (Table 3). In summary, these results show that in vivo glucose formation from pyruvate or glycerol is increased in fasting late pregnant rats and that glucose formation from alanine is augmented only when plasma

Table 4. Liver and Kidney Cortex Activities of Alanine and Aspartate Aminotransferase in Fasted 21-Day Pregnant Rats

	Alanine Aminotransferase*		Aspartate Aminotransferase*	
	Virgin	Pregnant	Virgin	Pregnant
LIVER				
Total	0.81 ± 0.05	0.46 ± 0.03†	2.03 ± 0.14	2.77 ± 0.23‡
Cytosolic fraction	0.40 ± 0.03	0.12 ± 0.01†	0.56 ± 0.06	0.59 ± 0.07
Mitochondrial fraction	0.18 ± 0.03	0.14 ± 0.02	1.06 ± 0.11	1.03 ± 0.12
KIDNEY CORTEX				
Total	0.12 ± 0.01	0.11 ± 0.01	1.57 ± 0.25	1.19 ± 0.16
Cytosolic fraction	0.04 ± 0.003	0.04 ± 0.004	0.42 ± 0.04	0.36 ± 0.03
Mitochondrial fraction	0.06 ± 0.003	0.07 ± 0.005	0.51 ± 0.04	0.45 ± 0.05

Values are mean ± SEM of 6–9 rats/group.

*Enzyme activities are expressed as μkat/g of fresh tissue.

Statistical comparisons between pregnant and virgin animals †$P < 0.001$, ‡$P < 0.05$.

concentrations are very high, indicating that its reduced endogenous availability in the fasted pregnant rat is a limiting factor for its conversion into glucose.

Incubations of Liver and Kidney Cortex Slices

To determine whether liver or renal gluconeogenesis was involved in the observed effects, glucose formation from alanine, pyruvate and glycerol was studied in liver and kidney cortex slices from 24-hour–starved 21-day pregnant rats and their virgin controls incubated in vitro, and results are presented in Tables 7 and 8. After 90-minute incubation of liver slices in the presence of 1 or 5 mmol/L substrates, glucose production always increased in proportion to the substrate concentration in the medium (Table 7). When alanine was used, values were significantly higher in liver slices from pregnant v control rats, whereas no differences were found in glucose formation when pyruvate or glycerol were the labeled substrate. The amount of substrate disappearing from the medium (ie, ^{14}C-substrate uptake) was significantly higher in liver slices of pregnant rats with alanine, whereas it was unchanged when pyruvate and glycerol were studied (Table 7).

When kidney cortex slices were incubated in vitro, glucose production from ^{14}C-alanine (5 mmol/L), ^{14}C-pyruvate (1 mmol/L, and ^{14}C-glycerol (1 and 5 mmol/L) was significantly increased in samples from the pregnant group compared with those from virgin animals. This increase in renal gluconeogenesis was not related to an enhanced disappearance of the substrates from the medium, as, although ^{14}C-substrate uptake always increased proportionally with the increase of the concentration of substrates in the medium, it did not differ in kidney cortex preparations from pregnant and virgin rats (Table 8). No changes were observed in the incorporation of the radioactive substrates to CO_2 or lactate (data not shown).

Table 5. ^{14}C-Glucose Formation in Fasted 21-Day Pregnant Rats After 1 mmol of Alanine ^{14}C (ul), Pyruvate ^{14}C (3), or Glycerol ^{14}C (ul)

Minutes After Tracer	dpm × 10^{-2} of ^{14}C-glucose/animal		
	2	5	10
Pyruvate ^{14}C (3)			
Virgin	260 ± 16	300 ± 20	670 ± 67
Pregnant	405 ± 24*	542 ± 57*	1059 ± 113†
Glycerol ^{14}C (ul)			
Virgin	813 ± 45	872 ± 35	2173 ± 263
Pregnant	1632 ± 441	1675 ± 260†	2120 ± 298
Alanine ^{14}C (ul)			
Virgin	247 ± 19	317 ± 14	708 ± 29
Pregnant	474 ± 46*	604 ± 33*	1051 ± 73‡

Values are Mean ± SEM of 6 to 10 rats/group.

Unanaesthetized rats were IV injected with the tracer (5 μCi/1 mmol/rat).

Statistical comparisons between pregnant and virgin animals: *$P <$ 0.001, †$P < 0.05$, ‡$P <$ 0.01.

DISCUSSION

Present results indicate that administration of moderate concentrations of pyruvate and glycerol enhanced glucose production in the starved pregnant rat, whereas no change was detected with alanine. This finding seems due to the limited availability of this amino acid (and presumably other gluconeogenic amino acids) in the pregnant rat, as glucose production with alanine was increased in liver and kidney cortex slices of pregnant rats incubated in vitro and also in vivo after receiving large amounts of this amino acid (1 mmol). Findings provide the first direct experimental evidence that deficiency of circulating alanine in fact leads to an impairment of gluconeogenesis in the starved pregnant rat, a hypothesis already proposed ten years ago by Freinkel and

Table 6. Plasma-Specific Activity of Alanine ^{14}C (ul) and Glycerol ^{14}C (ul) After 10 Minutes of 1 mmol Administration in 21-Day Pregnant Rats

	^{14}C-Substrate (dpm/mL)	Plasma Substrate Concentration (μmol/L)	^{14}C-Specific Activity (dpm/μmol)
Glycerol ^{14}C (ul)			
Virgin	4329 ± 347	3047 ± 251	1369 ± 89
Pregnant	2878 ± 461*	2446 ± 420	1226 ± 56
Alanine ^{14}C (ul)			
Virgin	4478 ± 452	7058 ± 844	650 ± 61
Pregnant	2709 ± 119†	4549 ± 371*	622 ± 21

Values are Mean ± SEM of 6 to 10 rats/group.

Unanaesthetized rats were IV injected with the tracer (5 μCi/1 mmol/rat).

Statistical comparisons between pregnant and virgin animals: *$P <$ 0.05, †$P <$ 0.01.

Table 7. ^{14}C-Glucose Formation and ^{14}C-Substrate Uptake by "in Vitro" Incubated Liver Slices From Virgin and Pregnant Rats

	μmol of Substrate/Hour · g Tissue			
	^{14}C-Glucose Formation		^{14}C-Substrate Uptake	
	Virgin	Pregnant	Virgin	Pregnant
Alanine ^{14}C (ul)				
1mmol/L	0.31 ± 0.07	0.56 ± 0.08*	2.63 ± 0.16	3.19 ± 0.18*
5 mmol/L	2.05 ± 0.27	3.63 ± 0.49*	14.10 ± 1.06	17.55 ± 0.84*
Pyruvate ^{14}C (3)				
1 mmol/L	1.25 ± 0.08	1.02 ± 0.11	6.23 ± 0.04	5.90 ± 0.18
5 mmol/L	6.54 ± 0.38	4.84 ± 0.96	27.85 ± 0.75	26.06 ± 0.41
Glycerol ^{14}C (ul)				
1 mmol/L	1.64 ± 0.31	1.66 ± 0.17	3.11 ± 0.43	3.22 ± 0.23
5 mmol/L	4.64 ± 1.16	5.94 ± 1.29	8.60 ± 1.34	12.32 ± 1.82

Values are Mean ± SEM of 6 to 9 rats/group.

Liver slices were incubated for 90 minutes in Krebs-Ringer bicarbonate buffer supplemented with one of the indicated ^{14}C-substrates.

*Statistical comparisons between pregnant virgin animals ($P < 0.05$).

associates.[8,26,27] A major factor possibly contributing to maternal reduction of circulating amino acids is their active transfer to the fetus throughout the placenta. It has recently been demonstrated that, as occurs in larger animals, in pregnant rats, placental alanine transfer to the fetus occurs very efficiently and against gradient.[28] Thus, the decreased availability of gluconeogenic amino acids in the starved, late pregnant rat reduces its actual gluconeogenetic capacity, although the pathway is activated, as shown by the efficient use of labeled pyruvate and glycerol for glucose synthesis. In the case of glycerol, this effect should be considered together with its augmented levels in maternal circulation, which indicate that glycerol constitutes a preferential gluconeogenetic substrate for the starved, late pregnant rat in comparison with the other substrates. In spite of enhanced maternal gluconeogenesis from certain substrates, glucose production is not sufficient to compensate for the enhanced glucose disappearance rate, which is known to occur at late pregnancy.[3] This effect is probably due to the high placental transfer of maternal glucose to the fetus.[29] In any event, it is evident that glucose utilization exceeds its synthesis in the starved mother, as shown by her marked hypoglycemia. In an attempt to prevent this hypoglycemia, and very probably as one of its consequences (hormonally mediated?), both the hepatic and renal gluconeogenetic pathways are activated, as shown by our in vitro experiments. This effect was specially clear for alanine in the liver (Table 7), and it was accompanied by an increase in alanine uptake in samples from pregnant rats. System A–mediated transport in the liver appears to constitute the rate-limiting step in alanine catabolism,[30–32] and this step may be activated in the pregnant rat liver, triggering the conversion of alanine into glucose wherever there is sufficient availability of the substrate. This possibility is supported by the fact that, unlike alanine, glucose production in liver slices from pregnant rats was not enhanced when either pyruvate or glycerol was the labeled substrate. This finding does not imply that utilization of these and other substrates on the in vivo hepatic gluconeogenetic activity is unchanged in the pregnant rat, since other factors such as increments in circulating concentration and in blood flow are partly responsible for their efficient conversion to glucose.

Reduced alanine aminotransferase activity in the liver of the starved pregnant rat is in agreement with prior reports in fed, pregnant animals.[33–35] This change should be considered together with the finding that, in the starved, pregnant rat, "in vivo" gluconeogenesis is not stimulated from low doses of alanine, while it is from equivalent doses of pyruvate, and

Table 8. ^{14}C-Glucose Formation and ^{14}C-Substrate Uptake by "in Vitro" Incubated Kidney Cortex Slices From Virgin and 21-Day Pregnant Rats

	μmols of Substrate/Hour · g Tissue			
	^{14}C-Glucose Formation		^{14}C-Substrate Uptake	
	Virgin	Pregnant	Virgin	Pregnant
Alanine ^{14}C (ul)				
1 mmol/L	0.29 ± 0.02	0.32 ± 0.02	3.64 ± 0.15	3.47 ± 0.23
5 mmol/L	0.49 ± 0.02	0.65 ± 0.04*	13.87 ± 1.98	14.57 ± 80
Pyruvate ^{14}C (3)				
1 mmol/L	0.87 ± 0.03	1.16 ± 0.09†	6.60 ± 0.002	6.61 ± 0.01
5 mmol/L	6.46 ± 0.45	7.38 ± 0.45	32.60 ± 0.04	32.56 ± 0.08
Glycerol ^{14}C (ul)				
1 mmol/L	2.49 ± 0.12	2.83 ± 0.08†	6.42 ± 0.3	6.39 ± 0.04
5 mmol/L	8.47 ± 0.22	11.05 ± 0.90†	19.64 ± 0.25	23.20 ± 1.52

Values are Mean ± SEM of 6 to 9 rats/group.

Kidney cortex slices were incubated for 90 minutes in Krebs-Ringer bicarbonate buffer supplemented with one of the indicated ^{14}C-substrates.

Statistical comparisons between pregnant and virgin animals: *$P < 0.01$, †$P < 0.05$.

also from high doses of either substrate. These findings indicate that alanine aminotransferase activity may be a rate-limiting step in the conversion of alanine ir to glucose in the presence of low, but not of high substrate concentrations.

The augmented glucose production from alanine, pyruvate, and glycerol in in vitro kidney cortex slices from 24-hour-starved pregnant rats indicates that this tissue also participates in the enhanced gluconeonesis of the starved mother. In this situation, there is hyperketonemia, which is correlated with urinary ammonia excretion.[1] Maternal acidosis may be responsible for the augmented renal gluconeogenesis, since these two factors have been shown to be interrelated in the nonpregnant rat through activation of key enzymes of the gluconeogenetic pathway.[36-40] The increased renal gluconeogenesis in the starved, pregnant rat may also be induced by her augmented catecholamine levels,[41,42] since it has been reported that in vitro renal gluconeogenesis is increased after the addition of catecholamines.[43,44] Moreover, these results confirm and extend our hypothesis that renal gluconeogenesis from alanine is functional and is under dietary[25] or hormonal control. It is noticeable that increased "in vitro" renal gluconeogenesis in the starved pregnant rat occurred without a parallel change in the substrate uptake (Table 8), suggesting that the effect results from altered channeling of the substrate taken up. Further studies will be required to substantiate this hypothesis.

Present results provide direct evidence that, in the starved, late pregnant rat, both hepatic and renal gluconeogenesis are activated, but reduced availability of certain substrates such as alanine limits their effectiveness. There must be adequate alanine amounts to reach a threshold level, causing increased gluconeogenesis from this substrate in the starved, late pregnant rat. Other metabolites seem to be used more efficiently as glucogenetic substrates in this condition. In this context, our findings indicate the important role of glycerol, which, in addition to its augmented circulating levels in the mother, is more efficiently converted into glucose than either pyruvate or alanine given in equivalent doses. It is proposed that the enhanced adipose tissue lipolysis in the starved, late pregnant rat[6,45] contributes actively to maternal glucose production by facilitating glycerol as a preferential gluconeogenetic substrate. The efficiency of this overall process is further attained due to the low placental permeability of glycerol in comparison with other substrates such as alanine,[29] which are indispensable to fulfill fetal needs.

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