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Research Article

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Glucose Turnover and Gluconeogenesis in Human Pregnancy

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Abstract

The rate of appearance (Ra) of glucose in plasma and the contribution of gluconeogenesis were quantified in normal pregnant women early (~ 10 wk) and late (~ 34 wk) in gestation. Their data were compared with those of normal nonpregnant women. Glucose Ra was measured using the $[U-^{13}C]$ glucose tracer dilution method. Gluconeogenesis was quantified by the appearance of 2H on carbon 5 and 6 of glucose after deuterium labeling of body water pool. Weight-specific glucose Ra was unchanged during pregnancy (nonpregnant, 1.89 ± 0.24 ; first trimester, 2.05 ± 0.21 ; and third trimester 2.17 ± 0.28 mg/kg-min, mean \pm SD), while total glucose Ra was significantly increased (early, 133.5 ± 7.2 ; late, 162.6 ± 16.4 mg/min; $P = 0.005$). The fractional contribution of gluconeogenesis via pyruvate measured by 2H enrichment on C-6 of glucose (45–61%), and of total gluconeogenesis quantified from 2H enrichment on C-5 of glucose (i.e., including glycerol [68–85%]) was not significantly different between pregnant and nonpregnant women. Inasmuch as total glucose Ra was significantly increased, total gluconeogenesis was also increased in pregnancy (early pregnancy, 94.7 ± 15.9 mg/min; late pregnancy, 122.7 ± 9.3 mg/min; $P = 0.003$). These data demonstrate the ability of the mother to adapt to the increasing fetal demands for glucose with advancing gestation. The mechanism for this unique quantitative adjustment to the fetal demands remains undefined. (*J. Clin. Invest.* 1997. 100:1775–1781.) Key words: pregnancy • glucose production • gluconeogenesis • $[^2H_2]O$ • $[^{13}C]$ glucose

Introduction

Glucose is the primary metabolic substrate for the growing conceptus, both for its energy needs as well as for carbon accretion (1). Since active gluconeogenesis in utero does not occur in most mammalian species studied, maternal glucose remains the entire source of glucose for the growing fetus (2, 3). The maternal and fetal glucose pools have been shown to be in

equilibrium in human and in other species, and a rapid transfer of glucose along a concentration gradient has been demonstrated. Using tracer isotope dilution methods, it has been shown that with advancing gestation there is an increase in the rate of appearance of glucose in the maternal circulation (2, 4–6), and that this increase is related to the increase in weight of the mother, and to the mass of the conceptus (7). Freinkel described pregnancy as a state of accelerated starvation, wherein there is a rapid decline in maternal glucose concentration during periods of fasting as well as an increase in circulating ketones (8). It was suggested that the decrease in glucose concentration may be due to the inability of the mother to sustain glucose production and gluconeogenesis in the presence of increased demands; specifically that contribution of gluconeogenesis may be insufficient for the increased rate of glucose production. Such inference was based upon the observed unchanged rate of urea excretion in early pregnancy (9), and decreased rate of urea synthesis after an overnight fast in late pregnancy (4). Using $[2,3-^{13}C_2]$ alanine tracer, we have previously observed a decreased incorporation of alanine C in glucose in humans in late gestation (10). Since the tracer C of alanine or lactate may be lost, in part via exchanges at the oxaloacetate level, the use of C-labeled gluconeogenic precursors results in underestimation of the contribution of gluconeogenesis (11). A number of approaches have been proposed to correct for the exchange or loss of the tracer at the level of oxaloacetate. Most of these approaches, however, are unsatisfactory due to the number of assumptions involved and the complex nature of laboratory analysis (11). Recently, deuterated water has been used to quantify gluconeogenesis in vivo (12, 13). Using this methodology, the contribution of gluconeogenesis to glucose production can be quantified during isotopic steady state by comparing the deuterium enrichment at C-6 or C-5 of glucose with that in body water. The rationale for these estimates has been presented previously (12, 13). Comparison of deuterium enrichment of H on C-6 of glucose originating from C-3 of pyruvate, and of H on C-5 of glucose originating from C-2 of triosephosphate with that of body water can give an estimate of gluconeogenesis from pyruvate and triosephosphate, respectively. The difference between the estimate from C-5 and C-6 is a measure of the contribution to gluconeogenesis of glycerol plus the extent that the equilibrium between deuterium in body water and methyl hydrogens of pyruvate is incomplete. In this study, we have used this approach to quantify gluconeogenesis in human pregnancy. The impact of advancing gestation on total glucose production and gluconeogenesis was examined by studying pregnant women in both early and late pregnancy.

Methods

Study population. Seven normal, nonobese women were recruited early in gestation to participate in the studies. Their clinical characteristics are displayed in Table I. None of the subjects had a family history of diabetes, were on any medications, or had any medical or obstetrical illness. They were studied in early gestation (mean 11.4 wk,

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Table I. Clinical Characteristics of the Study Population

	Gestation	Age	Prepregnancy wt	Study wt	Height	Body mass index
	wk	yr	kg	kg	cm	
Nonpregnant (<i>n</i> = 5)		30.0±7.8		67.7±11.3	167.4±4.4	0.24±0.03
Early pregnancy (<i>n</i> = 7)	11.4±1.7	31.1±4.1	64.0±8.6	65.8±7.9	165.7±3.1	0.24±0.03
Late pregnancy (<i>n</i> = 5)	34.6±1.5	31.0±4.9	59.0±5.0	75.7±10.1	168.2±2.5	0.26±0.03
Twin gestation	22	22	54.5	64.2	163	0.24

n = 7) and in late gestation (mean 34.6 wk, *n* = 5). One additional subject with twin gestation was studied twice at 22 and 34 wk, to examine the effect of increased fetal mass. All pregnant subjects delivered normal healthy infants at term gestation. Five nonpregnant normal women of a similar age group were studied as controls. Written informed consent was obtained from each subject and their spouse (when available) after fully explaining the procedure. The protocol was approved by the Institutional Review Board of University Hospitals of Cleveland. All studies were performed in the General Clinical Research Center at University Hospitals of Cleveland. [¹³C₆]glucose (99 atom % excess) and deuterium oxide (99.9 atom % D) were obtained from ISOTEC (Miamisburg, OH).

Study protocol. 3 d before the study, all subjects were placed on a diet containing at least 50% carbohydrates. The dietary compliance was evaluated from the records maintained by each subject. The nonpregnant subjects consumed an average of 2,130 kcal/d (range 1624–2723), 57.4% of which was from carbohydrate. Women in early gestation consumed an average of 2,435 kcal/d (1940–2977), 52% of which was from carbohydrate, while the calorie consumption of the late gestation group averaged 2,500 kcal/d (range 1926–3006) and consisted of 57% carbohydrate.

The subjects ate their last meals at 6:00 PM the evening before the day of study. To achieve ~0.5% enrichment of ²H in body water, they were given orally [²H₂]O, 5 g/kg body water, assuming total body water to be 55% of body weight. Because of the complaint of dizziness by two subjects, in later studies a smaller dose (~4 g/kg body water) of deuterated water was administered in three divided doses: the first dose was administered at bedtime (~11:00 PM), the second dose at around 3:00 AM, and the third dose after arriving at the Clinical Research Center at ~7:00 AM. 4 h later, the total rate of glucose appearance (Ra)¹ was measured by infusing [¹³C₆]glucose as prime constant rate infusion technique for 2 h. The rate of tracer infusion was 10 µg/kg·min. The prime consisted of ~90 min of tracer infusion. The dose of prime was adjusted depending upon the prevailing glucose concentration. The rate of tracer infusion was checked at the end of each study, as described (4, 6).

Plasma samples were obtained for [²H] enrichment in glucose 1 h after the last dose of deuterated water, and for the next 4 h (until 12:00 noon). Plasma samples to measure (m+6) enrichment of glucose were obtained at 15-min intervals starting 1 h after tracer infusion initiation.

Analytical methods. Deuterium enrichment of hydrogens on C-6 and C-5 of glucose was measured as described previously (13, 14). In brief, C-6 of glucose with its hydrogens, after preparatory isolation and purification, was cleaved by periodate oxidation to formaldehyde which, when condensed with ammonium hydroxide, forms hexamethylenetetramine. The latter was analyzed directly on a mass spectrometry system (HP 5970 equipped with an HP 5890 gas chromatograph; Hewlett-Packard Co., Palo Alto, CA). For estimation of deuterium enrichment on C-5 of glucose, after isolation of glucose from the

plasma, it was first converted to xylose, which was treated with periodic acid to cleave C-5 (13). The GC mass spectrometry conditions were as follows: a nonpolar polydimethyl siloxane stationary phase-bonded fused silica open-tubular column was used for GC mass spectrometry (AT-1; Alltech Assoc. Inc., Deerfield, IL). The column dimensions were 30 m × 0.54 mm I.D., and film thickness was 1.2 µm. Injection temperature was 170°C, oven initial temperature was 105°C for 6 min, final temperature was 230°C and ramp rate 45°C per min. The retention time of hexamethylenetetramine was ~3.2 min. Electron impact ionization (70 eV) was used, and ions *m/z* 140 and 141 were monitored using the selected-ion monitoring technique. Standard solutions of glucose of known enrichment were run along with unknowns to calibrate for instrumental variations.

The (m+6) enrichment (i.e., all carbons of glucose labeled with ¹³C) was measured using chemical ionization mass spectrometry as described from this laboratory previously (15).

Plasma glucose, free fatty acids, glycerol, and ketone levels were measured using enzymatic assays. Plasma insulin levels were measured using a double antibody radioimmunoassay. The lowest detection limit of this assay is 3 µU/ml. All of these analytical techniques have been reported from our laboratory previously (4).

²H enrichment of total body water was measured using zinc reduction method on an isotope ratio mass spectrometer (Dr. Catherine Leitch, Department of Pediatrics, Indiana University, IN).

Calculation. The rate of glucose appearance was calculated from the dilution of uniformly labeled glucose in plasma, as described (6).

The contribution of gluconeogenesis to glucose production was calculated by comparing the deuterium enrichment of hydrogens on carbon 5 and 6 of glucose with that of the body water. It is assumed that the methyl hydrogens of pyruvate (C-3) that form C-6 of glucose exchange with hydrogens in body water, so that ²H enrichment of hydrogens bound to C-3 of pyruvate or that of phosphoenolpyruvate becomes similar to that of water. Although this assumption has been evaluated in nonpregnant fasting subjects, and the exchange reaction has been found to be over 80% complete, the assumption has not been validated in human pregnant subjects. Based upon the data in this study, however, such an assumption appears to be valid in human pregnancy. C-5 of glucose is formed from C-2 of the triose phosphate. In the isomerization between dihydroxyacetone phosphate with glyceraldehyde-3-phosphate, the hydrogen on C-2 of glyceraldehyde-3-P is obtained from the water pool, and thus its enrichment is the same as that of body water. The two precursor pools of glucose, phosphoenolpyruvate and glyceraldehyde-3-phosphate, will have the same ²H enrichment on C-3 and C-2, respectively, as that of the body water. Therefore, comparison of deuterium enrichment of hydrogens on C-5 and C-6 of glucose with that of body water will give an estimate of the contribution of triose phosphate and pyruvate, respectively, to gluconeogenesis. The difference between the two estimates reflects the contribution of glycerol plus the extent to which the H exchange at the pyruvate level is incomplete. The rationale for these calculations have been discussed in detail previously (12, 13).

The contribution of GNG via pyruvate = 100 × (0.5 × [²H] enrichment on C-6/[²H] enrichment in water) %; contribution of total GNG = 100 × ([²H] enrichment on C-5/[²H] enrichment in water) %.

1. Abbreviation used in this paper: Ra, rate of appearance.

The enrichment on C-6 is multiplied by 0.5 because of two hydrogens on C-6. Total gluconeogenesis is calculated by multiplying the fractional contribution with total glucose Ra.

Statistical analysis. The three groups (nonpregnant, early, and late pregnant) were compared using Kruskal-Wallis one-way analysis of variance for unpaired data, and Wilcoxon rank sum test (2-tailed) was used when the data were not normally distributed. SPSS/PC+ statistical software system was used for analysis.

Results

Plasma glucose concentration after an overnight fast was higher in the nonpregnant group when compared with the pregnant subjects (Fig. 1). As the fasting continued, there was a significant decrease in plasma glucose concentration in all three groups. The decline in glucose was greatest in the late pregnancy group from 75 mg/dl to 65 mg/dl ($P = 0.0005$). As anticipated, with declining glucose concentration, there was an increase in betahydroxybutyrate concentrations (Fig. 2). A steady increase in plasma free fatty acid concentration was seen in the nonpregnant group. The increase in betahydroxybutyrate was greatest in late gestation group, even though there was no significant change in their free fatty acid concentration. Plasma glycerol concentrations were similar in the basal period in the three groups (nonpregnant 172.6 ± 24.9 ; early pregnant, 163.2 ± 31.9 ; late pregnant, 142.1 ± 29.1 $\mu\text{mol/liter}$). Plasma insulin concentrations during fasting were not significantly different, although the insulin levels were slightly higher in late as compared with early pregnancy.

Glucose Ra. The (m+6) enrichment of plasma glucose and the estimated rate of appearance of glucose (Ra) are displayed

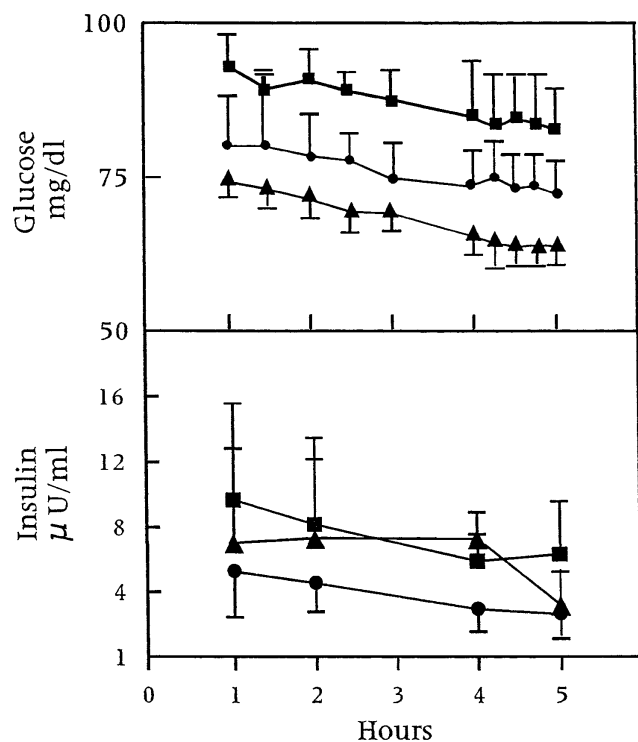


Figure 1. Changes in plasma glucose and insulin concentration during fasting. Pregnant early, late, and nonpregnant subjects received their last meal 14 h before time 0. Filled squares, nonpregnant; filled circles, early gestation; filled triangles, late gestation.

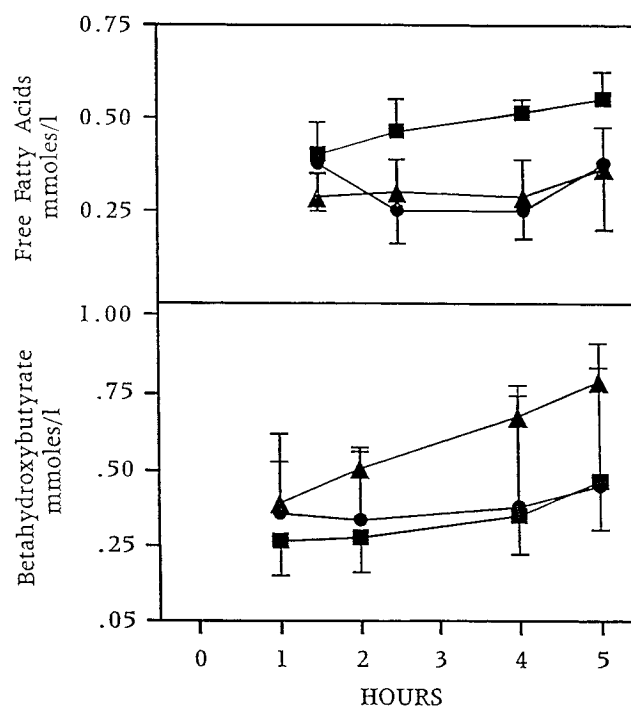


Figure 2. Plasma free fatty acid and betahydroxybutyrate concentrations. Pregnant early, late, and nonpregnant subjects received their last meal 14 h before time 0. Filled squares, nonpregnant; filled circles, early gestation; filled triangles, late gestation.

in Fig. 3. As shown, a steady state enrichment of tracer was achieved in all groups between 60 and 120 min of [$^{13}\text{C}_6$]glucose infusion. The weight-specific Ra of glucose increased slightly with advancing gestation as compared with early pregnancy. Since the weight of the mother had also increased during pregnancy, the total Ra of glucose ($\text{wt} \times \text{Ra}$) had significantly increased (early, 133.5 ± 7.2 ; late, 162.6 ± 16.4 mg/min; $P = 0.005$) with advancing gestation.

Gluconeogenesis in pregnancy. After administration of [$^2\text{H}_2$]O, a steady-state [^2H] enrichment on C-6 was observed by 4 h after the last dose of [$^2\text{H}_2$]O in all subjects. The data on a representative subject are displayed in Fig. 4. As shown, the ^2H enrichment on C-5 of glucose was also unchanged in the last hour before the [^{13}C]glucose infusion. The average deuterium enrichment of hydrogens on C-5 and C-6 of glucose in each subject, and the calculated contribution of gluconeogenesis estimated from the enrichments in C-5 and C-6, are displayed in Table II. The ratio of C6/C5, shown in the last column, represents the contribution of pyruvate to total gluconeogenesis. The contribution of pyruvate is underestimated by $\sim 20\%$ due to lack of complete equilibrium between methylhydrogens of pyruvate and body water. In the nonpregnant women after an ~ 16 h fast, total gluconeogenesis contributed 50–77% (average 67%) to total glucose production, while gluconeogenesis via pyruvate represented 36–48% (average 44%) of glucose Ra. In early pregnancy, contribution of total gluconeogenesis was slightly increased to an average of 72%, and it was further increased in late gestation to 76%. Of interest, there was a greater increase in pyruvate contribution to glucose during pregnancy when compared with the nonpregnant state, repre-

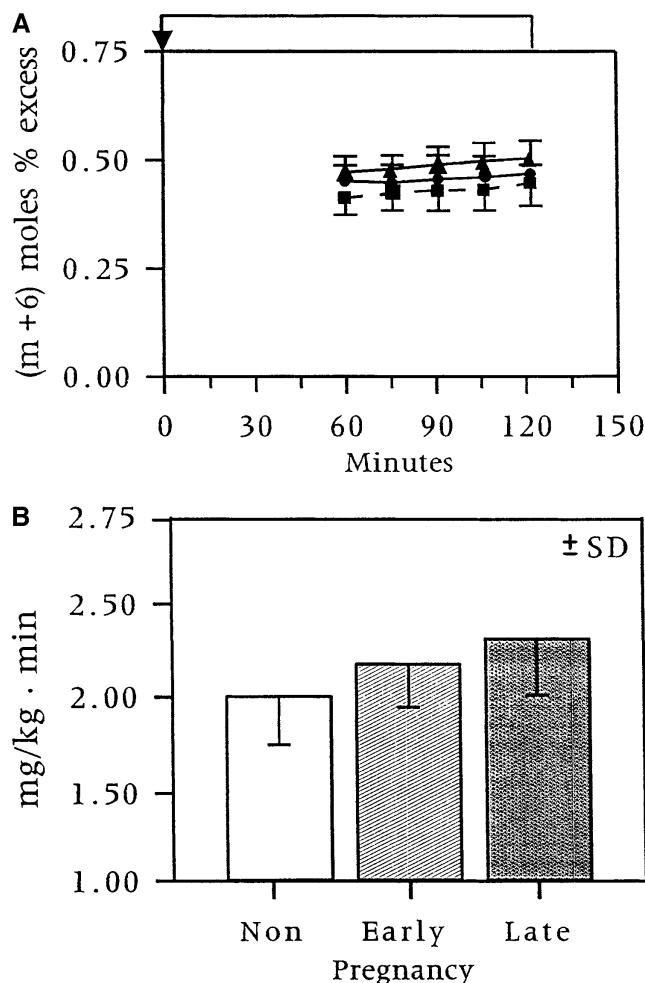


Figure 3. (A) (m+6) enrichment of plasma glucose after prime constant rate [$U-^{13}C$]glucose infusion and (B) the calculated rate of glucose appearance (glucose Ra) in pregnant and nonpregnant women. Filled squares, nonpregnant; filled circles, early gestation; filled triangles, late gestation.

senting 61% in early gestation (range 38–82%), and 52% (range 38–62%) in late gestation. These changes, however, did not reach statistical significance. The contribution via pyruvate to gluconeogenesis represented by the ratio of $[^2H]$ enrichments on C-6 and C-5 was the same in nonpregnant subjects and those studied in late gestation, and was increased in early gestation.

In one subject with twin pregnancy, glucose Ra was 2.12 and 2.34 mg/kg·min at 22 and 34 wk gestation, respectively, while the contribution of gluconeogenesis (C-5) was 67% and 84%.

A linear correlation was observed between plasma betahydroxybutyrate and fractional contribution of gluconeogenesis in each group and in all subjects together [$y = 48.3 + 3.74x$ (%), $r = 0.61$, $P = 0.011$] (Fig. 5).

Discussion

Glucose turnover during pregnancy. The data in this study reporting decreasing plasma glucose concentration with advancing gestation and no change in the weight-specific rate of glu-

cose turnover, are similar to those reported previously by us (2, 4, 6, 10) and by other investigators (16, 17). Inasmuch as the maternal weight increased by 16–18% during pregnancy, the total rate of glucose turnover has also increased by this magnitude during late gestation. The mechanism of the decrease in plasma glucose concentration during fasting in pregnancy remains unclear; it has been attributed to the increase in volume of distribution of glucose (18) or a feto-placental glucose steal phenomenon (19, 20). The data in this study confirm the previous data and suggest that with advancing gestation, commensurate with the increasing demands by the growing conceptus, there is an increase in total rate of glucose turnover (7).

Estimation of gluconeogenesis in vivo. Several approaches have been applied for quantification of the contribution of gluconeogenesis to total glucose appearance in vivo (11). The estimates based upon transfer of C from amino acids, lactate, or pyruvate into glucose result in underestimation of gluconeogenesis due to exchange of the tracer carbon with unlabeled carbon in the hepatic oxaloacetate pool, which is a common intermediate for both oxidation by the Krebs cycle and gluconeogenesis (21). Although several methods have been proposed for correcting this metabolic exchange of the tracer carbon, most of these are cumbersome and complicated (11, 22–26). More recently, Rothman et al. (27), Petersen et al. (28), and Magnusson et al. (29) have quantified gluconeogenesis indirectly by subtracting the rate of hepatic glycogenolysis, measured by magnetic resonance imaging, from the tracer isotope-measured rate of glucose production. Other methods have used glycogen-labeling technique (30) and mass isotopomer distribution analysis using [$U-^{13}C$]glucose tracer (31). All of these methods have resulted in estimates of gluconeogenesis in normal healthy subjects during the postabsorptive state to be in the range of 25 to 60%. Deuterium labeling of body water pool, used here, results in $[^2H]$ labeling of gluconeogenic precursors in the liver and kidney. The appearance of deuterium in hydrogens on C-6 of glucose formed from C-3 of pyruvate, and hydrogens on C-5 of glucose formed from C-2 of triose-phosphate, gives an estimate of gluconeogenesis via pyruvate and pyruvate plus glycerol, respectively (13). Inasmuch as C-3 pyruvate labeling depends upon the equilibration with the wa-

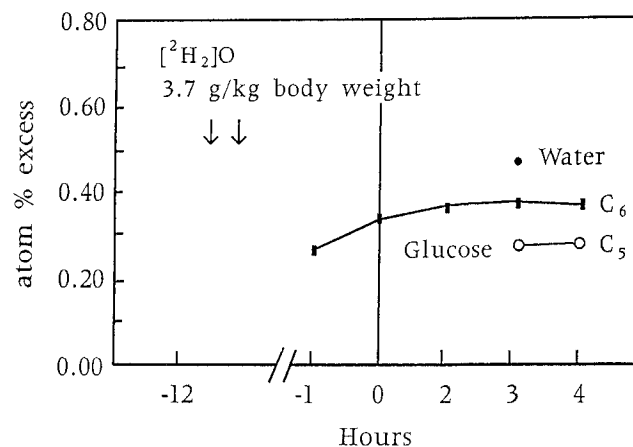


Figure 4. Time course of 2H enrichment on carbon 5 and 6 of glucose after administration of oral $[^2H_2]O$ in a pregnant subject. $[^2H_2]O$ was given as 3.7 g/kg body wt in divided doses. 2H enrichment of water is also displayed.

Table II. [^2H] Enrichment in Body Water, at C-5 and C-6 of Glucose and Estimates of Gluconeogenesis in Pregnancy

		% ^2H Enrichment			% Gluconeogenesis		% (0.5 \times C-6)/C-5
		Water	C-5	C-6	C-5	C-6	
Nonpregnant	1	0.491	0.380	0.467	77.4	47.6	61.4
	2	0.593	0.305	0.578	51.4	48.7	94.8
	3	0.276	0.210	0.263	76.1	47.6	62.6
	4	0.291	0.215	0.248	73.9	42.6	57.7
	5	0.279	0.160	0.203	57.3	36.4	63.4
Mean \pm SD		0.386 \pm 0.147	0.254 \pm 0.088	0.352 \pm 0.162	67.2 \pm 11.9	44.6 \pm 5.2	67.9 \pm 15.1
Pregnant							
Early gestation*	1	0.461	0.355	0.440	77.0	47.7	62.0
	2	0.473	0.275	0.357	58.1	37.7	64.9
	3	0.427	0.220	0.477	51.5	55.9	108.6
	4	0.283	0.240	0.463	84.8	81.8	96.5
	5	0.325	0.255	0.373	78.5	57.4	73.1
	6	0.313	0.245	0.370	78.3	59.1	75.5
	7	0.317	0.220	0.318	69.4	50.2	72.3
Mean \pm SD		0.371 \pm 0.079	0.258 \pm 0.047	0.400 \pm 0.060	71.1 \pm 12.1	55.7 \pm 13.6	78.9 \pm 17.1
Late gestation	1	0.453	0.270	0.340	59.6	37.5	63.0
	2	0.265	0.220	0.330	83.0	62.3	75.1
	3	0.258	0.210	0.288	81.4	55.8	68.6
	4	0.258	0.190	0.244	73.6	47.3	64.2
	5	0.307	0.255	0.355	83.1	57.8	69.6
Mean \pm SD		0.308 \pm 0.08	0.229 \pm 0.03	0.311 \pm 0.05	76.1 \pm 10.0	52.1 \pm 9.8	68.1 \pm 4.8

*Same subjects (1–5) were studied in early and late gestation.

ter pool, and that equilibration in pregnancy has not been documented, the appearance of ^2H on C-6 glucose may result in an underestimation of gluconeogenesis.

As discussed previously, simultaneous measurement of deuterium labeling of carbons 2, 5, and 6 of glucose can give an estimate of total gluconeogenesis, and that from pyruvate without having to wait for isotopic steady state (13). In this study, because all subjects were already in isotopic steady state with respect to C-5 and C-6 of glucose, we elected not to measure the ^2H enrichment on C-2 of glucose. Estimate of ^2H enrichment on C-2 of glucose in one subject gave results similar to that in body water (data not shown).

Estimates of fractional contribution of gluconeogenesis after an overnight fast in nonpregnant women via pyruvate (36–49%) and from pyruvate plus glycerol (51–77%) are in the same range as those reported previously.

Gluconeogenesis in pregnancy. Previous data on gluconeogenesis during pregnancy are conflicting due to differences in methodologies, and due to species differences. Data from human studies (10, 32) using ^{13}C labeled alanine show that even though the weight-specific rate of alanine and glucose turnover in human pregnancy remains unchanged, the fractional contribution of alanine C to glucose is decreased. The decreased contribution of alanine C to glucose was attributed to the decreased contribution of alanine to the lactate/pyruvate pool, possibly due to decreased activity of the deamination/transamination step (33, 10). These studies are in contrast to those in the pregnant rat, where an increased turnover and catabolic rate of alanine was reported by Pastor-Anglada and co-workers (34–36), and increased rate of turnover of lactate and its incorporation into glucose was reported by Valcarce et al. (37). In

addition, increased activity of key gluconeogenic enzymes phosphoenolpyruvate carboxykinase and hexose biphosphatase has been observed in the liver of pregnant rats at term gestation (37). These conflicts in the reported data are partly due to the difference in species with different fetal/maternal mass ratios, and partly due to the difference in the tracer isotopes used. Pastor-Anglada and co-workers used ^2H or ^3H -labeled alanine, resulting in a high estimate of alanine turnover due to loss of beta hydrogen during transamination/deamination (38, 39). Other investigators have used C-labeled tracers of alanine

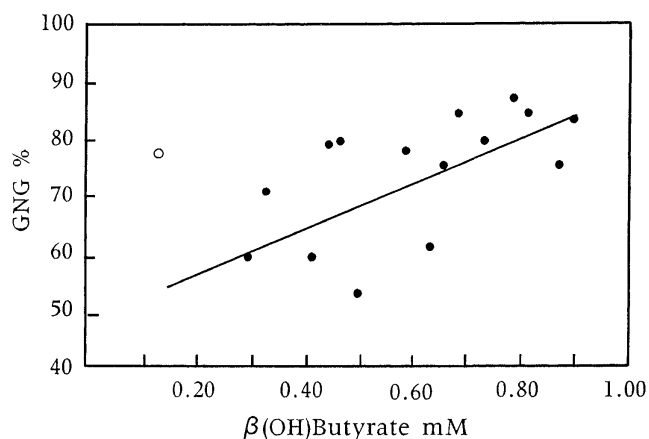


Figure 5. Correlation between plasma betahydroxybutyrate concentration and the contribution of gluconeogenesis in all subjects. Subject represented by (o) was excluded from calculation of correlation. $y = 48.3 + 3.74x$ (%); $r = 0.61$, $P = 0.011$.

and lactate that would lead to underestimation of alanine and lactate turnover due to recycling of tracer C (38). In addition, as stated earlier, C-labeled tracers will lead to underestimation of gluconeogenesis due to exchanges in the TCA cycle.

Other investigators have attempted to quantify gluconeogenesis in pregnancy by measuring the rate of glucose C recycling. Gilbert et al. (40) estimated glucose C recycling in the pregnant guinea pig from the difference in the rates of glucose turnover as measured by $[6\text{-}^3\text{H}]\text{glucose}$ and $[\text{U-}^{14}\text{C}]\text{glucose}$ tracer. They showed that glucose C recycling increased from 0% at 40 d gestation to 20% at term gestation (~ 63 d). In contrast, Ogata et al. (41) did not observe any change in glucose C recycling in their studies of pregnant rats late in gestation.

All of the studies cited above have the limitations inherent with hydrogen and carbon-labeled substrates: increased loss of tracer with hydrogen and/or exchange of tracer with carbon. This study, using deuterium-labeled water, obviates most of the problems, and thus provides a more precise estimate of gluconeogenesis in pregnancy. As shown, there was a small but not significant increase in the contribution of gluconeogenesis from pyruvate (C-6) during pregnancy. The change in total gluconeogenesis (C-5) during pregnancy was even smaller. By comparing the same subjects during early and late gestation, we had hoped to demonstrate maximal changes in gluconeogenesis, if any, during pregnancy. No significant difference in total contribution of gluconeogenesis or pyruvate to glucose production could be seen. Using the observed effect and standard deviation, sample size estimate showed that, to demonstrate a statistically significant difference in total gluconeogenesis ($P = 0.05$, 2-tail) with a power of 80%, it would require 24 subjects per group in nonpregnant versus early pregnancy studies, and 150 subjects per group in early versus late pregnancy studies. The smallest difference that could be shown with a power of 70% given the sample size and the standard deviation would have been $1.5 \times \text{SD}$.

The significant correlation between betahydroxybutyrate levels and the contribution of gluconeogenesis is anticipated because of the demonstrated role of ketogenesis in providing energy for gluconeogenesis (42–44). It is of interest that the increased betahydroxybutyrate levels in late pregnancy were not associated with increased fatty acid levels, suggesting independent regulation of ketogenesis.

Pregnancy and accelerated starvation. The data from this study and from those previously reported in human pregnancy (2, 4–6, 10, 16, 17) suggested that the concept of accelerated starvation of pregnancy should be reevaluated. As originally proposed, accelerated starvation in pregnancy was based upon changes in circulating substrate after a brief fast (8). As noted here and in previous studies, a brief fast in pregnancy is associated with a greater decline in plasma glucose and a greater increase in betahydroxybutyrate levels. In contrast to the situation in fasting, however, kinetic data in this study show an increase in total rate of glucose production, i.e., $\text{wt} \times \text{Ra}$ (early pregnancy, 133.5 ± 7.2 mg/min; late pregnancy, 162.6 ± 16.4 mg/min; $P = 0.005$). In addition, there was a corresponding increase in total contribution of gluconeogenesis (C-5) (early pregnancy, 94.7 ± 15.9 mg/min vs. late pregnancy, 122.7 ± 9.3 mg/min; $P = 0.003$), even though the fractional contribution of GNG remained unchanged. Furthermore, previous data have shown an increase in respiratory quotient (6) and a decrease in urea synthesis (4) during late gestation. All of these are in conflict with the concept of accelerated starvation.

In summary, in this study we have applied the deuterated water method to quantify gluconeogenesis in human pregnancy. Our data show that commensurate with increasing demands of late gestation, there is an increase in total glucose production and gluconeogenesis. Such an increase in both total glucose production and gluconeogenesis is contrary to the concept of accelerated starvation of pregnancy.

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