The Diabetes in Early Pregnancy Study

β-Hydroxybutyrate levels in type 1 diabetic pregnancy compared with normal pregnancy

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OBJECTIVE — The objective was to assess relationships between β-hydroxybutyrate (β-OHB) level and pregnancy outcome in human pregnancy in light of the fact that high levels of β-OHB cause malformations and growth retardation in in vitro studies.

RESEARCH DESIGN AND METHODS — We analyzed β-OHB in prospectively collected specimens from the National Institute of Child Health and Human Development–Diabetes in Early Pregnancy Study, in gestational weeks 6–12 in diabetic (n = 204–239) and nondiabetic (n = 316–332) pregnant women.

RESULTS — Levels of β-OHB in diabetic women were 2.5-fold higher than in nondiabetic pregnant women at 6 weeks' gestation and declined to 1.6-fold above nondiabetic women by 12 weeks' gestation ($P < 0.0001$ at all times). β-OHB was positively correlated with glucose levels ($P < 0.0001$) in diabetic mothers, probably reflecting degree of diabetic control. β-OHB correlated inversely with glucose ($P < 0.0003$) (gestational week 6 only) in nondiabetic mothers, possibly reflecting caloric intake. β-OHB tended to be lower (not higher) in diabetic and nondiabetic mothers with malformed infants or pregnancy losses, but the difference was not statistically significant. β-OHB in diabetic mothers at 8, 10, and 12 weeks correlated inversely with birth weight (P = 0.004–0.02), even after adjusting for maternal glucose levels. β-OHB levels were also generally lower in diabetic mothers of macrosomic infants, and week 12 ultrasound crown–rump measurements were inversely related to β-OHB levels.

CONCLUSIONS — The 1st trimester β-OHB is significantly higher in diabetic than nondiabetic pregnant women. In both groups, β-OHB tended to be lower, not higher, in mothers who had a malformed infant or pregnancy loss. β-OHB was inversely related to crown–rump length and birth weight. The modest β-OHB elevation in the 1st trimester of reasonably well-controlled diabetic pregnancy is not associated with malformations, probably because β-OHB levels causing malformations in embryo culture models are 20- to 40-fold higher. The mechanism of the β-OHB association with impaired fetal growth is unknown.

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Abbreviations: β-OHB, β-hydroxybutyrate; DIEP, Diabetes in Early Pregnancy.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Ketone body metabolism is important in the diabetic pregnancy for both mother and fetus. Experimental models of diabetic pregnancy (1) indicate that maternal ketonemia may be implicated in the pathogenesis of congenital malformations. Serum ketone levels are elevated with both poor diabetic control and poor nutrition, and ketone formation is accelerated in pregnancy (2,3). Because ketones are freely transported to the fetus and serve as a source of energy, they could spare other fuels that contribute to macrosomia. Indeed, the causes of macrosomia in the infant of the diabetic mother are still controversial and could involve all maternal fuels (4,5).

The Diabetes in Early Pregnancy (DIEP) Study, a multicenter, cohort study of type 1 diabetic women and normal control women throughout pregnancy, was designed to answer questions related to causes of spontaneous abortions and malformations (6,7). The study reported an increased risk of spontaneous abortion as the maternal glycemia rises above the normal range. Normal control women had a spontaneous abortion rate of 16%; diabetic women showed a rate that rose from 9% when the glucose control was in the normal range to >45% when the glycemic level was markedly elevated (6). In this study, diabetic women had a twofold increased risk, compared with a normal population, of bearing a malformed infant, which could not be related to the maternal glucose level (7).

Given the DIEP study design, it was possible to ask ancillary questions related to abnormalities of fetal growth in the diabetic and normal patient populations. This study has reported that the risk of macrosomia increases with increased maternal postprandial glucose levels (8). β-Hydroxybutyrate (β-OHB) levels were also measured in these women throughout the 1st trimester to assess the potential importance of metabolites of fatty acid oxidation in pregnancy outcome. In this report, the DIEP research group analyzes the β-OHB levels obtained prospectively in the multicenter study to determine the relationship between β-OHB levels and the etiology of spontaneous abortion, malformation, and neonatal macrosomia.

RESEARCH DESIGN AND METHODS

— In the DIEP study, diabetic control women were recruited from research groups from Cornell University, Brigham & Women's Hospital (Harvard University), Northwestern University, the University of Pittsburgh, and the University of Washington. The National Institute of Child Health and Human Development served as the data and coordinating center. The study design has been described in detail elsewhere (6,7,9). Briefly, diagnosis of pregnancy was made during the week of missed menses, and diabetic subjects were immediately hospitalized for 2–5 days for monitoring of metabolic control and entry testing. Other than teaching the techniques of self-monitoring of blood glucose a minimum of four times daily, a standard approach to the management of diabetes was not imposed, nor were fixed goals for glycemic control mandated. After discharge, diabetic subjects were monitored and had blood samples drawn at weekly clinic visits from weeks 5 to 12 after the last menstrual period and monthly thereafter. Control subjects were recruited in the same fashion as diabetic women (6–9) and had blood drawn at biweekly intervals. Control subjects were screened for gestational diabetes at 26 weeks according to the criteria established by the Second International Gestational Diabetes Workshop (10).

Subjects included in the β-OHB analysis were DIEP early-entry diabetic and control women who had their samples analyzed prospectively for fasting plasma glucose, nonfasting glucose, glycated hemoglobin, and β-OHB from gestational weeks 6 to 12. A total of 204–239 type 1 diabetic women and 316–332 normal control women were studied at the four study intervals. Blood samples were collected serially at weeks 6, 8, 10, and 12 in the diabetic and the normal control groups. The majority of pregnancy losses (included in this analysis are spontaneous abortions, defined as pregnancy loss before 20 weeks, and fetal deaths, defined as from 20 weeks on) occurred before week 12 of gestation. However, we included in the analysis pregnancy loss up to 34 weeks of gestation if the woman had samples drawn for analysis at weeks 6, 8, 10, and 12.

Venous overnight fasting plasma glucose was measured in each of the five centers using either a Yellow Springs International Instruments glucose analyzer (Yellow Springs, OH) or a Beckman Autoanalyzer (Palo Alto, CA). Glycosylated hemoglobin was analyzed in a central laboratory at the University of Washington using the thiobarbituric acid calorimetric assay (11).

Plasma for β-OHB was analyzed using a direct kinetic assay of β-OHB dehydrogenase reaction on a Gilford Model 203S according to standard procedures (12). The reference normal nonpregnant control range for β-OHB is 60–250 μmol/l.

Table 1—*Patient characteristics*

	Control	Diabetic	Statistical significance
n	428	380	
Age (years)	29.7 ± 3.9	28.0 ± 4.1	$P < 0.001$
Height (cm)	163.9 ± 7.29	163.4 ± 7.4	NS
Weight (kg)	62.8 ± 12.3	63.1 ± 9.7	NS
Race			
White	391 (91.4)	355 (93.4)	
Nonwhite	37 (8.6)	24 (6.3)	$\chi^2 = 1.54$, $P = 0.2$
Unknown	0 (0)	1 (0.3)	
Income (U.S. $)			
<10,000	15 (3.5)	22 (5.8)	
10,000–20,000	47 (11.0)	67 (17.6)	
20,000–25,000	49 (11.4)	58 (15.3)	$\chi^2 = 21.2$, $P < 0.001$
25,000–35,000	101 (23.6)	87 (22.9)	
≥35,000	203 (47.4)	125 (32.9)	
Unknown	13 (3.0)	21 (5.5)	
Maternal education			
High school graduate	20 (4.7)	75 (19.7)	
Some college	107 (25.0)	126 (33.2)	
College graduate	123 (28.7)	101 (26.6)	$\chi^2 = 73.2$, $P < 0.001$
Some graduate school	82 (19.2)	32 (8.4)	
Completed graduate school	96 (22.4)	45 (11.8)	
Unknown	0 (0)	1 (0.3)	
Number of completed pregnancies			
0	297 (9.4)	246 (64.7)	
1	105 (24.5)	110 (29.0)	$\chi^2 = 2.1$, $P = 0.3$
2	23 (5.4)	23 (6.1)	
3	3 (0.7)	1 (0.3)	
Number of prior fetal losses			
0	344 (80.4)	256 (67.4)	
1	62 (14.5)	79 (20.8)	$\chi^2 = 20.7$, $P < 0.001$
2	16 (3.7)	28 (7.4)	
≥3	6 (1.4)	17 (4.5)	
Cigarette use			
None	378 (88.3)	322 (84.7)	
<1 pack/day	28 (6.5)	35 (9.2)	$\chi^2 = 4.1$, $P = 0.13$
≥1 pack/day	13 (3.0)	19 (5.0)	
Unknown	9 (2.1)	4 (1.1)	
Alcohol consumption			
None	56 (13.1)	124 (32.6)	
Yes	364 (85.0)	252 (66.3)	$\chi^2 = 43.7$, $P < 0.001$
Unknown	8 (1.9)	4 (1.1)	

Data are means ± SD or *n* (%).

Statistical analysis

Because the data were non-normally distributed, we mainly relied on nonparametric statistical tests. For example, we applied the Spearman's test to testing significance of correlations. When we compared between two groups, we applied the two-sample Wilcoxon nonparametric test. We also applied parametric tests after taking logarithmic transformations of the data. Both parametric and nonparametric tests produced comparable results. We chose to report *P* values based on nonparametric tests, because they tended to be conservative and robust.

Table 2—*Fasting β-OHB levels throughout the 1st trimester*

	6 weeks	8 weeks	10 weeks	12 weeks
Control women (n = 332)	0.13 ± 0.13	0.11 ± 0.09	0.10 ± 0.07	0.10 ± 0.09
Diabetic women (n = 239)	0.32 ± 0.31	0.21 ± 0.20	0.17 ± 0.14	0.16 ± 0.15
P value	0.0001	0.0001	0.0001	0.0001
Change from previous week				
Control women	—	−0.02 ± 0.14	−0.01 ± 0.07	0.003 ± 0.10
Diabetic women	—	−0.09 ± 0.32†	−0.03 ± 0.23*	−0.004 ± 0.16
P value	—	0.003	NS	NS

*$P < 0.06$; †$P < 0.001$.

RESULTS — Patient characteristics are shown in Table 1. Diabetic and control subjects were similar in mean height, weight, race, parity, and smoking. However, diabetic subjects were younger, less affluent, less well educated, had more previous fetal losses, and drank less alcohol than control women.

Fasting β-OHB levels in the diabetic and normal control women are shown in Table 2. β-OHB levels in the diabetic women were significantly higher ($P < 0.0001$) at all the weeks studied, initially elevated 2.5-fold at week 6 and then declining monotonically to 1.6-fold by week 12. The decrease observed in the diabetic group between weeks 6 and 12 is coincident with the improvement in glycemic control reported previously in these subjects (7). β-OHB levels did not change during the 1st trimester in the control subjects. In addition, the β-OHB levels within diabetic women tracked over time. Table 3 shows that the correlations of values from week to week in the diabetic group were very high ($P < 0.0005$).

The nonparametric (Spearman's) correlations of β-OHB levels with plasma glucose and glycosylated hemoglobin levels throughout the 1st trimester are shown in Table 4. Nondiabetic women had a significantly negative correlation between β-OHB and glucose at week 6 ($r = -0.22$, $P = 0.0003$). A significantly negative correlation between β-OHB and glycosylated hemoglobin was also observed in the normal pregnant women at week 6 ($r = -0.17$, $P = 0.04$). The inverse relationships persisted at weeks 8, 10, and 12 for glucose and for 8 and 10 weeks for glycosylated hemoglobin, but at those times the relationships were weaker and no longer statistically significant.

In diabetic subjects, β-OHB levels are positively associated with plasma glucose (Table 4). The positive correlation is statistically significant at all weeks studied in the 1st trimester. There was no correlation between glycosylated hemoglobin and β-OHB levels at any week studied in the diabetic group.

β-OHB levels in the presence and absence of malformation in infants of normal and diabetic women are shown in Table 5. Normal women bearing a malformed infant tended to have lower β-OHB levels at 6, 8, and 10 weeks compared with those having normal infants. Likewise, diabetic women having a malformed infant (n = 11) also had slightly lower β-OHB levels at all weeks of gestation than did those having normal infants, but statistical significance was borderline at best.

With respect to pregnancy loss, mean β-OHB concentrations and range of values observed in the diabetic and control women tended to be lower in women with pregnancy loss (Table 6). None of the differences were significant except for one instance at week 12 in normal subjects.

Regarding relationships with birth weight (Table 7), a significant negative correlation was observed between the β-OHB levels at weeks 8, 10, and 12 and the birth weight in infants of diabetic mothers (Table 7). This negative relationship persisted after adjusting for plasma glucose level (Table 7), which we have previously reported as influencing birth weight (8)—i.e., a glucose level >120 mg/dl significantly increased the risk for the birth of a large-for-gestational-age infant. The negative associations seen between β-OHB and birth weight in normal pregnancies were not statistically significant when adjusted for glucose (Table 7).

An analysis of variance was not performed because it would be considering β-

Table 3—*Spearman's correlations for the diabetic women*

Week	n	r	P
6 vs. 8	164	0.4	0.0001
6 vs. 10	167	0.3	0.0001
6 vs. 12	152	0.3	0.0004
8 vs. 12	163	0.4	0.0001
8 vs. 12	155	0.4	0.0001
10 vs. 12	157	0.5	0.0001

Table 4—*Spearman's correlations of β-OHB levels with glycemic status throughout the 1st trimester*

	6 weeks	8 weeks	10 weeks	12 weeks
Control women				
β-OHB vs. glucose				
n	251	280	257	264
r	−0.22	−0.06	−0.01	−0.07
P value	0.0003	0.3	0.9	0.4
β-OHB vs. glycosylated hemoglobin				
n	135	92	81	92
r	−0.17	−0.03	−0.12	0.11
P value	0.04	0.8	0.3	0.3
Diabetic women				
β-OHB vs. fasting glucose				
n	162	183	191	184
r	0.28	0.26	0.16	0.23
P value	0.0004	0.0003	0.03	0.001
β-OHB vs. glycosylated hemoglobin				
n	124	146	155	147
r	0.07	−0.03	0.01	0.02
P value	0.5	0.7	0.9	0.08

Table 5—*β-OHB levels in mothers of normal and malformed infants*

	Control women				Diabetic women			
	6 weeks	8 weeks	10 weeks	12 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Normal infants								
n	319	325	308	316	229	212	209	195
β-OHB (mmol/l)	0.13 ± 0.13	0.11 ± 0.09	0.10 ± 0.07	0.10 ± 0.09	0.32 ± 0.32	0.21 ± 0.20	0.17 ± 0.15	0.17 ± 0.15
Malformed infants								
n	8	7	8	8	9	12	11	11
β-OHB (mmol/l)	0.12 ± 0.09	0.08 ± 0.04	0.07 ± 0.03	0.10 ± 0.10	0.19 ± 0.18	0.18 ± 0.20	0.10 ± 0.06	0.14 ± 0.09
P value	0.95	0.50	0.20	0.70	0.08	0.2	0.08	0.9

Data are *n* or means ± SD.

OHB as the dependent variable (outcome) as it was influenced by malformation rates and time (independent variables). We also did an analysis looking at combined effects of several variables. For malformations in the diabetic group, change in β-OHB over time was not a significant predictor, but being a diabetic woman was significant. When the two variables were entered together, neither was significant, which may be a problem of sample size. For macrosomia, both change in β-OHB (decreasing) and status (diabetic) were significant predictors of macrosomia. When they were analyzed together, only having diabetes was a significant predictor. We did not do this analysis for pregnancy loss because the time variable was not testable, as too many losses occurred in the first weeks of pregnancy.

To determine if β-OHB levels were related to infant macrosomia—defined as the >90th percentile of birth weight adjusted for race, sex, and gestational age—β-OHB levels were examined in mothers of infants above and below those birth-weight cut points. The results (Table 8) show lower β-OHB levels among mothers of macrosomic infants, which are seen in control women at 8 weeks' gestation and in diabetic women at 8, 10, and 12 weeks' gestation. These results are consistent with the negative association of birth weight with β-OHB level, especially in diabetic pregnancy (Table 7).

Because the DIEP study has previously reported (13) that the 12-week crown–rump length assessed by ultrasound is significantly shorter in up to 30% of the infants of diabetic mothers who are born with birth defects, we examined the association of crown–rump length with plasma β-OHB level (Table 9). In normal control subjects, the β-OHB levels measured at 6, 8, 10, and 12 weeks of gestation show consistent negative correlations at all times with crown–rump length (r = 0.15–0.23; P = 0.02–0.0007), with the strongest correlation occurring at week 6. The diabetic women also show a negative correlation, which is significant for the β-OHB level at weeks 8 and 12 (r = 0.08–0.18; P = 0.03–0.02). We concluded in our previous report (13) that among type 1 diabetic subjects who were moderately well-controlled at conception, statistically significant but mild early fetal growth delay was present but did not appear to be useful clinically in predicting congenital malformations. The β-OHB level in the present report did not add to the predictive value of the week-12 ultrasound.

CONCLUSIONS— The DIEP Study was performed to examine pregnancy outcome among type 1 diabetic and nondiabetic women, all of whom had intensive metabolic monitoring in the 1st trimester. We have previously reported on the relationship between glycemic status and congenital malformations, spontaneous abortion, and infant macrosomia (6–8). We have not reported the associations of these outcome variables with plasma ketone-body levels in this cohort.

In general, plasma ketone-body levels (β-OHB, acetoacetate, and acetone) reflect the rate of fatty acid oxidation in the body, primarily the liver. The rate of fatty acid oxidation is primarily controlled by the levels of plasma insulin and free fatty acids. When insulin levels are low, ketogenesis is accelerated; when insulin levels are high, ketogenesis is inhibited. Three clinical conditions are classically associated with accelerated ketogenesis, two generally applicable to mammalian physiology and one specifically applicable to pregnancy. The two generally applicable conditions are caloric deprivation and diabetes. In both instances, cells are deprived of glucose in association with a diminished level or insufficient action of insulin, thereby accelerating ketogenesis. In pregnancy, the same physiology applies, but

Table 6—*β-OHB levels in mothers experiencing pregnancy loss (spontaneous abortion or fetal death)*

	Control women				Diabetic women			
	6 weeks	8 weeks	10 weeks	12 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Deliveries								
n	291	301	298	317	202	194	200	197
β-OHB (mmol/l)	0.13 ± 0.14	0.11 ± 0.09	0.10 ± 0.07	0.11 ± 0.09	0.33 ± 0.33	0.20 ± 0.18	0.17 ± 0.14	0.17 ± 0.15
Losses								
n	36	31	18	7	37	30	18	7
β-OHB (mmol/l)	0.11 ± 0.07	0.11 ± 0.09	0.07 ± 0.05	0.06 ± 0.04	0.26 ± 0.21	0.26 ± 0.32	0.14 ± 0.17	0.14 ± 0.08
P value	0.4	0.6	0.06	0.02	0.4	0.1	0.2	0.8

Data are *n* or means ± SD.

Table 7—*Spearman's correlations of β-OHB levels with percentile group of birth weight adjusted for race, sex, and gestational age*

	Normal women				Diabetic women			
	6 weeks	8 weeks	10 weeks	12 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Adjusted for sex of offspring, gestational age, and race								
n	288	298	295	315	202	194	200	197
r	−0.09	−0.10	−0.03	−0.006	−0.12	−0.21	−0.17	−0.16
P value	0.14	0.09	0.60	0.90	0.1	0.004	0.02	0.02
Adjusted for plasma glucose levels								
n	251	280	267	294	162	183	191	184
r	−0.07	−0.09	−0.05	−0.02	−0.09	−0.023	−0.23	−0.16
P value	0.24	0.13	0.39	0.67	0.25	0.001	0.04	0.03

ketogenesis is especially accelerated in the 3rd trimester due to a decline in maternal glucose levels (attributed mainly to fetal glucose utilization) (14); a parallel fall in plasma insulin levels (15); and enhanced mobilization of fatty acids caused by the lipolytic hormones of pregnancy (14–16). Overall, this metabolic realignment can progress to the "accelerated starvation" of pregnancy should caloric intake be deficient (3,14–17). Hyperketonemia in pregnancy has been implicated in the pathogenesis of congenital malformations (1) and in mental impairment of the offspring of ketonemic mothers (18–20).

In contrast to the 3rd trimester, when the tendency to "accelerated starvation" is at its maximum, plasma ketone-body physiology has not been studied in detail in the 1st trimester, either descriptively or with respect to pregnancy outcome. The only report we are aware of on this subject is that of Montelongo et al. (21), who studied mean plasma ketone-body levels in the 1st, 2nd, and 3rd trimesters of gestational diabetic and overtly diabetic (type 1) pregnant women. These authors found plasma free fatty acid levels elevated 50–100% above control values in the 1st trimester (as well as in the 2nd and 3rd). Plasma β-OHB levels were three- to fivefold above normal in gestational and type 1 diabetic pregnancy, again in all three trimesters. The number of subjects in Montelongo et al.'s study was small (n = 33), such that examination of associations of metabolic variables with perinatal outcome was not feasible and measurements were not made at specific times in the 1st trimester.

The present report confirms and extends that of Montelongo et al. (21), showing that for each of the weeks in which β-OHB levels were measured in the 1st trimester, levels in the diabetic group were significantly higher than in the control group. The 1st trimester β-OHB levels in our cohort (2.5- to 1.6-fold that of the control group) were not as elevated as in the report of Montelongo et al. and decreased between 6 and 12 weeks in contrast to Montelongo et al. (21), where the β-OHB elevations persisted throughout the three trimesters. These differences are likely due to the level of glycemic control, because blood glucose levels in Montelongo's type 1 diabetic subjects remained elevated twofold throughout the three trimesters in contrast to the rapid improvement in glycemic control in DIEP subjects in the 1st trimester (7).

The hypothesis that good glycemic control (i.e., lower blood glucose levels in diabetic women) is associated with lower ketone-body levels was affirmed in our study, which found a positive relationship between plasma glucose and β-OHB in the diabetic subjects. As mentioned above, a crucial element in attaining diabetic control is the provision of adequate levels of circulating insulin. In contrast, in our nondiabetic pregnant control subjects, β-OHB levels are negatively associated with fasting plasma glucose levels. In this instance, the inverse relationship may reflect the accelerated fat oxidation during caloric deprivation that is especially likely to occur in association with the nausea and erratic food intake characteristic of early pregnancy. It is noteworthy that the inverse association of glucose and glycosylated hemoglobin with β-OHB is statistically significant only at gestational week 6.

Regarding congenital malformations and ketonemia, a number of embryo culture studies have shown that hyperketonemia is associated with congenital malformations (1,22–24). However, very high doses of β-OHB are necessary to cause malformations.

Table 8—*Plasma β-OHB levels in mothers of normal versus macrosomic infants*

	Control women				Diabetic women			
	6 weeks	8 weeks	10 weeks	12 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Normal infants								
n	246	257	254	272	139	137	139	139
β-OHB (mmol/l)	0.13 ± 0.14	0.11 ± 0.09	0.10 ± 0.07	0.11 ± 0.10	0.33 ± 0.28	0.22 ± 0.19	0.18 ± 0.14	0.17 ± 0.15
Macrosomic infants								
n	42	41	41	43	63	57	61	58
β-OHB (mmol/l)	0.12 ± 0.11	0.08 ± 0.05	0.09 ± 0.07	0.09 ± 0.05	0.33 ± 0.42	0.17 ± 0.04	0.15 ± 0.15	0.15 ± 0.14
P value	0.1	0.005	0.2	0.1	0.3	0.04	0.03	0.04

Data are *n* or means ± SD.

Table 9—*Spearman's correlation coefficients between β-OHB level in the mother and fetal length (crown–rump length)*

	n	*r*	*P* value
Control women			
Week 6	208	−0.23	0.0007
Week 8	219	−0.16	0.02
Week 10	215	−0.20	0.004
Week 12	226	−0.15	0.02
Diabetic women			
Week 6	158	−0.08	0.3
Week 8	158	−0.18	0.02
Week 10	158	−0.09	0.3
Week 12	156	−0.18	0.02

For instance, Horton et al. (1) used 32 mmol/l concentrations to produce malformations; Freinkel et al. (22) found minor malformations at 8 mmol/l and major malformations at 16 mmol/l β-OHB concentrations. Major malformations were also observed by the latter group when 8 mmol/l β-OHB and 600 mg/dl glucose concentrations were maintained in embryo culture (22). By contrast, DIEP subjects with the highest β-OHB levels were not at such levels. In fact, β-OHB levels rarely reach such high levels, even in diabetic ketoacidosis. Buchanan et al. (23) found that adding glucose or β-OHB to sera derived from acutely insulin-treated sera from diabetic pregnant rats contributed little to the teratogenic effect of the sera per se. Thus, it is not surprising that we found no elevation in β-OHB level in mothers having a malformed offspring.

It is difficult to say whether the trend toward lower plasma β-OHB levels in normal and diabetic pregnant women with malformed offspring compared with unaffected offspring is biologically meaningful. The vulnerable period for dysmorphogenesis occurs early in this interval (weeks 6–8), as described by Mills et al. in 1979 (25). Recently, redox status has been implicated in the cause of congenital malformations (26). Ordinarily, hyperglycemia is associated with diabetic embryopathy (24), but we found no association between hyperglycemia and congenital malformation in the diabetic DIEP subjects monitored and treated from 6 weeks' gestational age onward. On the other hand, the late-entry subjects who were more poorly controlled did have an increase in congenital malformations (7). Thus, the pathogenesis of congenital malformations in reasonably well-controlled diabetic pregnancy remains an open question.

Regarding spontaneous abortions or pregnancy losses, we have previously reported higher mean fasting and postprandial plasma glucose concentrations in affected pregnancies (6). In this report, we confirm that pregnancy loss is a frequent phenomenon, occurring in approximately 15% of all clinically recognized pregnancies (6). At least 20% of pregnancies are lost before clinical recognition, and a previous report (27) showed that there may be an interval up to 2–3 weeks between fetal demise and clinical recognition of pregnancy loss. The majority of losses reported in this article followed the same trend. However, no increase in β-OHB levels was seen in normal or diabetic pregnancy ending in spontaneous abortion or fetal death. In fact, the trend was toward lower levels in fetal losses. It is uncertain if there is any biological significance associated with this trend.

Data are still sparse on the relationship between maternal ketone levels and subsequent birth weight. The DIEP study has previously reported (8) that the 1st trimester nonfasting glucose levels are significantly correlated with subsequent infant birth weight; however, when the nonfasting 1st trimester glucose level is subjected to multivariate analysis, the 3rd trimester nonfasting glucose level emerges as the strongest variable to correlate with infant birth weight (8). The DIEP study measured β-OHB levels only in the 1st trimester. Nonetheless, the 1st trimester β-OHB levels are significantly negatively correlated with subsequent birth weight in infants of diabetic mothers. That this observation remains when nonfasting glucose level is controlled for is intriguing, because the plasma glucose and β-OHB levels are positively rather than negatively associated in the diabetic pregnancy. The negative association of β-OHB with birth weight of infants of type 1 diabetic mothers raises the possibility of a deleterious effect of ketone bodies per se on infant growth, as has been seen at higher ketone-body levels in embryo culture (22,28,29).

The examination of relationships of β-OHB levels with ultrasound-determined crown–rump length offers another opportunity to examine relationships of ketone level and growth, but in a narrower time frame. In this instance, the strong negative association of β-OHB with crown–rump length by ultrasound at 12 weeks' gestation in subjects with normal ketone-body levels and the less strong association in diabetic women favors the view that β-OHB with higher ketone-body levels is an indicator of caloric sufficiency/insufficiency, reflecting its inverse association with plasma glucose rather than a direct toxic effect. Further studies of β-OHB levels throughout pregnancy are needed to determine the relationship between β-OHB and birth weight, the interrelationships with other fuels, and the apparent discrepancy between inverse associations of β-OHB with growth in normal pregnancy at 12 weeks and in diabetic pregnancy at term. Until definitive studies are conducted, the prudent treatment protocol for both normal and diabetic women is to provide sufficient calories to maintain the pregnancy and, hence, prevent starvation ketosis. In the case of diabetic pregnancy, careful attention must be given to avoid hyperglycemia-induced ketogenesis, which is associated with high rates of fetal mortality.

In summary, we have presented a detailed and extensive investigation of plasma ketone levels in the 1st trimester. We confirm a β-OHB elevation early in the 1st trimester in diabetic pregnancy, which improves with therapy in subsequent weeks but is not normalized by week 12. We also find the expected positive association of β-OHB and glucose levels in the diabetic pregnancy, which indicates that glycemic control is an important determinant of β-OHB levels in diabetic pregnancy. An inverse association of β-OHB with glucose—at least at the earliest time point in the normal pregnancy—and an inverse association of β-OHB with crown–rump length may reflect maternal caloric sufficiency. No associations between β-OHB and malformations, spontaneous abortions, or fetal deaths were detected in our study, possibly because the in vitro concentrations of β-OHB required to cause malformations or growth retardation in the rat embryo culture model must be 20- to 40-fold higher than observed in this study. These results support the clinical goal that near normalization of glycemic status remains a central objective to attain ideal pregnancy outcome in the type 1 diabetic mother (30) and encourage further research on the relationship of diverse maternal fuels, including β-OHB, with fetal growth and development.

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