Metabolic Profiles in Closely Controlled Diabetic Pregnancies during the Third Trimester

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Summary. In 5 closely controlled pregnant diabetics (duration of pregnancy 237–266 days) and 5 pregnant non-diabetics (duration of pregnancy 210–278 days) 4-hourly blood samples were taken throughout a 24 h period and analyzed for blood glucose, lactate, pyruvate, 3-hydroxybutyrate and acetoacetate, plasma non-esterified fatty acids (NEFA), glucagon and cortisol. 24 h urine specimen was analyzed for total catecholamines and 4-hydroxy-3-methoxymandelic acid. There were few significant differences in concentrations of metabolites and hormones in the two groups at any time, although the variations about the mean was usually greater in the diabetics. Thus for blood glucose in diabetics, mean value was 4.4 mmol/l, coefficient of variation 43%; in non-diabetics 4.1 mmol/l and 10% respectively. Mean plasma 3-hydroxybutyrate in diabetics was 0.47 mmol/l, coefficient of variation 55%; in non-diabetics 0.44 mmol/l and 37% respectively. Plasma non-esterified fatty acid levels were significantly higher in the diabetics (0.47 mmol/l) than in the non-diabetics (0.26 mmol/l). Coefficients of variation were 46% and 33% respectively. Two conclusions can be drawn; first, when near normal mean values for blood glucose are achieved, other metabolite and hormone levels are also near normal; second, even when the available means for diabetic control, strict diet and insulin-mixtures twice daily, are used at their maximum, metabolism in diabetics is more unstable than in non-diabetics.

Key words: Diabetic pregnancy, metabolic profile, diabetes mellitus, insulin therapy, metabolic control, glucagon, cortisol, growth hormone, ketone bodies, blood glucose, blood lactate, blood pyruvate, plasma non-esterified fatty acids.

Insulin treatment of the diabetic represents a form of replacement therapy. Subcutaneously injected insulin, however, is not a physiological substitute for insulin produced by the β -cells; its release into the circulation will be independent of the blood glucose concentration and the other metabolic, nervous and hormonal factors which regulate the normal release of insulin. Furthermore, insulin released from the subcutaneous tissues will reach the systemic circulation directly, bypassing the portal vein and liver. The balance between the effects of insulin on the liver and the peripheral tissues in insulin treated diabetics will therefore differ from that in normal individuals.

In diabetic pregnancy normalization of metabolism is probably of major importance for the outcome [4, 12]. In most studies blood glucose normalization has been the goal, and only recently have other metabolites been studied [5, 6].

The present study was undertaken to evaluate to what extent normalization of metabolism is possible in intensely controlled, hospitalized, insulin-dependent, pregnant diabetics.

Materials and Methods

Patients

The diabetic subjects, all with long-standing juvenile diabetes, were taking part in a special regimen for pregnant diabetics [9]. On this regimen they were admitted to the ward in the 33rd week of pregnancy; delivery usually took place 1–3 weeks before term. The study was performed during this period. A standard diet of 3 main meals and two smaller meals was given. Blood glucose concentration was determined 4 times daily (0800, 1200, 1600, 2000 h) and insulin was given twice daily, before breakfast at 0800 h and at 1730 h before the main evening meal, as a mixture of intermediate-acting (isophane) and soluble insulin, the dose being adjusted daily. During the hospital stay the patients were encouraged to exercise regularly.

Table 1. Data on the diabetic subjects

Patient ^a number	Age (years)	Parity	Duration of diabetes (years)	Daily ^b insulin dose (i. u.)	Duration of pregnancy at sampling (days)	Duration of pregnancy at delivery (days)	Birth weight of baby (g)
1	27	0	20	24+ 8 16+ 8	248	259	3210
2	20	0	9	40+20 24+12	249	269	4300
3	22	2	12	44+24 12+16	260	265	3250
4	34	2	23	32+16 24+20	237	263	3140
5	29	0	11	16+ 4 8+ 8	266	271	3520

^a Patient 1–4 had simple retinopathy only, patient 5 no signs of angiopathy

^b Insulin was given twice daily. The dose of isophane insulin is written first, the dose of soluble insulin second

Table 2. Data on the non-diabetic subjects

Patient number	Age (years)	Parity	Duration of pregnancy at sampling (days)	Duration of pregnancy at delivery (days)	Birth weight of baby (g)	Reason for hospitalization
6	27	1	210	242	2030	Moderate hypertension
7	27	1	215	275	3100	Low total estrogen in urine
8	27	1	278	281	3850	Transverse lie
9	29	1	271	281	3500	Transverse lie, placenta praevia
10	22	0	215	240	2450	Antepartum haemorrhage

Table 1 shows the age, parity, duration of diabetes, duration of the pregnancy at the time of the study, insulin dosage and further course of pregnancy in the 5 diabetic subjects. In Table 2 are shown the age, parity, reason for hospitalization, duration of pregnancy at the time of the study, and further course of pregnancy in the 5 control subjects. None of the subjects were taking any medication at the time of study. All gave informed consent to the study, and all later gave birth to healthy babies.

On the day of the study blood samples were taken through an indwelling venous cannula at 0800 (fasting), 1200, 1600, 2000, 2400, 0400 and 0800 h. The samples at 0800 and 1200 h were taken before breakfast and lunch, at 1600 h one hour after their mid-afternoon snack, at 2000 h 2½ h after their evening meal, and at 2400 h 3 h after their evening snack.

The non-diabetics had usual hospital meals, no snacks at 1500 and 2100 h, and were not given any dietary instructions.

Methods

Glucose was determined by a glucose oxidase method adapted for Auto-analyzer. Blood samples for lactate and pyruvate determinations were immediately mixed with equal volumes of ice-cold perchloric acid (1 mol/l), and samples were kept frozen (–20 °C) until analyzed. The concentrations of lactate and pyruvate were determined by the Biochemica Test Combination (Boehringer Mannheim GmbH). Ketone bodies (3-hydroxybutyrate and acetoacetate) were determined on the perchloric acid extracts by

an enzymatic method [17]. The plasma concentration of non-esterified fatty acids (NEFA) was determined by a titrimetric method [13]. Plasma cortisol was determined by radioimmunoassay (Diagnostic Products Corporation, Los Angeles). The concentration of 4 hydroxy – 3 methoxy mandelic acid (HMMA) and catecholamines in urine were determined by routine methods, HMMA by spectrophotometry after oxidation with periodate, catecholamines by fluorimetry after conversion to lutein derivatives. Plasma glucagon was determined by radioimmunoassay [8, 14]. NOVO Research Institute kindly supplied the pancreas specific antiserum (K 964), glucagon for standards and (¹²⁵I) glucagon. All samples were assayed in triplicate. Unspecific glucagon activity was determined after treatment with charcoal (Norit A) to remove true glucagon, and subtracted from total glucagon to give true glucagon. Coefficient of variation of the assay was 9.4% at glucagon concentration of 60 ng/l. The lower detection limit was approximately 20 ng/l.

Student's t-test has been used to test for statistical significance, using $p < 0.05$ as the level of statistical significance.

Results

The results are given in Table 3 and 4. Blood glucose values did not differ significantly at any time, although the variations were greater in the diabetics,

Table 3. Concentration of various metabolites in blood in pregnant diabetic (D) and non-diabetic subjects (N)

Time		0800	1200	1600	2000	2400	0400	0800
Glucose mmol/l	D	4.6±1.7 (2.7–6.2)	3.9±1.2 (2.0–5.1)	4.1±0.9 (3–5)	5.0±1.8 (2.6–7.0)	3.8±2.2 (1.8–7.4)	4.1±2.4 (2.4–7.4)	5.3± 3.2 (2.5–10.4)
	N	3.4±0.3 (2.9–3.7)	4.4±0.5 (4.0–5.2)	4.5±0.6 (4.0–5.2)	4.6±0.3 (4.1–5.0)	4.5±0.5 (4.0–5.3)	3.7±0.3 (3.3–4.0)	3.4± 0.3 (3.1– 3.6)
Lactate mmol/l	D	0.9±0.3 (0.6–1.3)	1.0±0.2 (0.7–1.2)	0.9±0.2 (0.7–1.2)	1.1±0.3 (0.6–1.4)	0.9±0.3 (0.6–1.3)	0.9±0.2 (0.7–1.1)	0.9±0.2 (0.7–1.3)
	N	0.9±0.1 (0.7–1.1)	0.9±0.3 (0.6–1.3)	1.1±0.3 (0.7–1.6)	1.1±0.4 (0.8–1.5)	0.9±0.2 (0.7–1.1)	1.1±0.3 (0.6–1.3)	0.9±0.4 (0.5–1.2)
Pyruvate mmol/l	D	0.06±0.02 (0.04–0.08)	0.07±0.01 (0.05–0.08)	0.07±0.01 (0.05–0.09)	0.08±0.03 (0.05–0.11)	0.07±0.02 (0.05–0.1)	0.07±0.01 (0.07–0.08)	0.07±0.01 (0.06–0.09)
	N	0.07±0.01 (0.06–0.08)	0.09±0.04 (0.05–0.14)	0.09±0.03 (0.07–0.14)	0.09±0.03 (0.05–0.13)	0.08±0.02 (0.04–0.1)	0.1 ±0.03 (0.05–0.12)	0.07±0.01 (0.05–0.08)
Lactate/ Pyruvate ratio	D	14.8± 1.7 (12.9–16.7)	14.2± 2 (11.7–15.7)	13.6± 2.4 (10–16)	13.0± 2.3 (10–16)	12.7± 0.5 (12 –13.3)	11.9± 1.8 (10 –13.8)	12.5± 2.4 (10 –16.3)
	N	12.8± 0.8 (11.6–13.8)	10.5± 1.6 (7.9–12)	11.4± 0.9 (10–12.2)	12.0± 2.5 (10–16)	12.2± 3.2 (8.8–17.5)	10.9± 0.07 (10 –12)	12.2± 4.5 (8.3–17.1)
NEFA mmol/l	D	0.77 (0.18–1.38)	0.36 (0.22–0.51)	0.43 (0.21–0.72)	0.40 (0.17–0.57)	0.29 (0.09–0.40)	0.38 (0.16–0.58)	0.67 (0.25–1.05)
	N	0.42 (0.28–0.73)	0.22 (0.13–0.32)	0.24 (0.19–0.41)	0.18 (0.10–0.23)	0.27 (0.21–0.34)	0.24 (0.13–0.30)	0.22 (0.19–0.28)
3-Hydroxy- butyrate mmol/l	D	0.81 (0.42–1.47)	0.31 (0.24–0.48)	0.46 (0.22–0.78)	0.39 (0.22–0.61)	0.37 (0.12–0.78)	0.39 (0.16–0.61)	0.53 (0.31–0.96)
	N	0.49 (0.31–0.62)	0.46 (0.38–0.65)	0.38 (0.28–0.59)	0.44 (0.20–0.61)	0.47 (0.18–0.73)	0.44 (0.26–0.63)	0.39 (0.16–0.49)
Aceto- acetate mmol/l	D	0.26 (0.12–0.39)	0.11 (0.06–0.16)	0.13 (0.06–0.17)	0.16 (0.06–0.20)	0.10 (0.06–0.25)	0.10 (0.07–0.15)	0.17 (0.09–0.29)
	N	0.18 (0.09–0.26)	0.16 (0.08–0.24)	0.09 (0.06–0.15)	0.13 (0.09–0.17)	0.11 (0.06–0.19)	0.14 (0.08–0.19)	0.16 (0.08–0.28)

Values are given as mean ± SD, range in parenthesis

the coefficient of variation being 43% versus 10% in the non-diabetics. Figure 1 shows the individual curves for the two groups. Mean blood glucose through the whole period was 4.4 mmol/l for the diabetics, 4.1 in the non-diabetics.

Plasma NEFA were on the whole higher in the diabetic group, the difference did not, except at 2000 h, reach statistical significance. Again the coefficient of variation was higher for the diabetics, 46% versus 33%. Figure 2 shows the individual curves for the two groups. The mean NEFA for the 24 h was 0.47 mmol/l for the diabetics, 0.26 mmol/l for the non-diabetics; this difference is significant ($p < 0.0005$).

Three of the non-diabetic patients had relatively short duration of pregnancy (210–215 days) at the time of testing, and two had a long duration (271 and 278 days). As shown in figure 1 and 2 this did not seem to influence their blood glucose and NEFA levels.

Ketone body levels (3-hydroxybutyrate and acetoacetate) were remarkably similar in the two groups, again the coefficient of variation was greater in the diabetics, 55% versus 43% in the non-diabetics.

Blood lactate and pyruvate levels and their variation were similar in the two groups. The lactate/pyruvate ratios tended to be higher in the diabetics than in the non-diabetics, mean values for the whole period 13.2 and 11.7 respectively; the difference is statistically significant ($p < 0.005$).

Plasma glucagon levels and variation did not differ significantly in the two groups. Plasma cortisol also tended to be higher in the diabetics; the differences are not significant.

Urinary excretion of catecholamines and HMMA did not differ in the two groups. Total urine catecholamines was 379 nmol/24 h and 306 nmol/24 h in diabetics and non-diabetics respectively; urinary HMMA 14 µmol/24 h and 18 µmol/24 h, respectively.

Fig. 1. Individual blood glucose values. Upper panel: diabetics; lower panel: non-diabetics. The curves are numbered for each patient as in Tables 1 and 2

Fig. 2. Individual plasma NEFA values. Upper panel: diabetics; lower panel: non-diabetics. The curves are numbered for each patient as in Tables 1 and 2. At 2000 h the mean NEFA level in the diabetics is significantly higher than for the non-diabetics, ($p < 0.05$)

Table 4. Plasma glucagon and cortisol in pregnant diabetic (D) and non-diabetic (N) subjects

a) Time		0800	1200	1600	2000	2400	0400	0800
Glucagon ng/l	D	46 ± 9 (34–56)	56 ± 10 (42–69)	58 ± 17 (39–76)	57 ± 9 (47–69)	57 ± 3 (53–61)	48 ± 9 (36–59)	52 ± 10 (44–68)
	N	43 ± 12 (23–55)	51 ± 18 (26–74)	48 ± 14 (32–66)	54 ± 12 (41–66)	47 ± 14 (33–65)	49 ± 11 (33–58)	49 ± 11 (36–60)
Cortisol nmol/l	D	992 ± 100 (825–1075)	818 ± 236 (413–1025)	853 ± 133 (718–999)	734 ± 201 (463–1000)	590 ± 216 (350–875)	804 ± 356 (348–1198)	949 ± 159 (700–1098)
	N	982 ± 239 (583–1176)	797 ± 181 (560–1014)	637 ± 207 (313–817)	524 ± 119 (333–574)	536 ± 248 (193–767)	714 ± 280 (320–1140)	825 ± 379 (405–1140)

Results are given as mean ± SD, range in parenthesis. No differences at any time point between the two groups are significant ($p > 0.05$)

Discussion

The present study gives an indication of what can be obtained under optimal conditions by our presently available means in the treatment of diabetics in pregnancy. In practical terms the results are satisfactory, as the program has reduced the perinatal mortality

significantly, to 3% (5/170) in those followed from before the 28th week of pregnancy [9]. This figure has to be compared to the total perinatal mortality in Norway which in 1976 was 1.32%.

The study, like Buckle et al.'s [3] demonstrates that normalization of blood glucose, which has been the parameter for adjusting insulin dose, diet and

general management also gives fairly normal blood levels for other metabolites and hormones. Non-esterified fatty acids are the exception; this may be of particular importance as it has been suggested that increased NEFA levels may explain adiposity in infants of diabetic mothers [11]. None of the 5 infants born to the diabetic mothers in the present study, however, were overweight.

There is a greater variation in the blood levels of many metabolites in the diabetic group, illustrating that their metabolism is more unstable than in non-diabetics, and that completely physiological conditions cannot be obtained with subcutaneously injected insulin.

Better stabilization in diabetic pregnancies can, however, probably be achieved by a glucose controlled insulin infusion system. At present this can only be used for short periods such as during labour [10].

It must be noted that the non-diabetic controls in this study are not normal controls; they were all hospitalized for various complications of pregnancy. Also they differed from the diabetics in duration of pregnancy. The influence of these factors is difficult to evaluate, but it is probable that if the non-diabetics had been completely normal and of the same duration of pregnancy, the variations in their blood metabolite levels might have been even smaller.

Our blood samples were drawn at 4 – hourly intervals. Others [1, 2, 5, 6, 7, 15, 16] have demonstrated fluctuations which will not have been detected using our sampling schedule. Typical is the postprandial morning blood glucose, and postinsulin injection lactate rise found in insulin treated diabetics [1, 2]. Quite low blood sugars were reached in some of the diabetic subjects, the lowest values being 1.8 mmol/l. It is possible that some even lower values occurred during the sampling intervals. It is, however, our general experience that severe symptomatic hypoglycemia is relatively rare in pregnant diabetics on this program [9].

Alberti et al. [1, 2] have recently published studies showing poor metabolic control, sometimes with very abnormal metabolic rhythms, in insulin dependent diabetics under more ordinary circumstances where no special effort to optimize the control was used. The regimen used for pregnant diabetics may be a model for improving metabolic control in diabetics in general.

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