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Diurnal Variations in Blood Intermediary Metabolites in Mild Gestational Diabetic Patients and the Effect of a Carbohydrate-Restricted Diet

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Summary. Twenty-four-hour metabolic profiles were performed in the third trimester of pregnancy in seven non-diabetic women (group A) and in two groups of mild gestational diabetics, at diagnosis (group B, seven patients) and after treatment with a 150-g carbohydrate diet (group C, seven patients). Mean 24-h blood metabolite levels (± SD) in groups A, B and C were: *glucose:* 4.65 ± 0.82, 5.35 ± 3.06 and 5.40 ± 1.7 mmol/l; *lactate:* 1.05 ± 0.18, 1.14 ± 0.42 and 0.78 ± 0.22 mmol/l; *alanine:* 0.31 ± 0.03, 0.31 ± 0.10 and 0.27 ± 0.07 mmol/l; *ketone bodies:* 0.11 ± 0.04, 0.19 ± 0.05 and 0.26 ± 0.15 mmol/l. Glucose levels were not significantly different between the groups. Ketone body levels were elevated in the diabetics prior to treatment ($p < 0.01$) and rose higher on diet. Lactate levels were reduced on the diet ($0.05 < p < 0.10$). Abnormalities in the concentrations of total blood ketone body levels in gestational diabetics may be detrimental to fetal development and therapy should be designed to minimise changes in intermediary metabolites.

Key words: Pregnancy in diabetes, gestational diabetes, diabetic diet, metabolism, gluconeogenesis, lipid mobilisation, glycolysis, glucose, lactate, alanine, total ketone bodies, acetoacetate, 3 hydroxybutyrate.

Gestational diabetes has been associated with increased perinatal mortality and morbidity rates [1–4] although the rise in mortality is not confirmed in all studies [5]. Although hyperglycaemia in the mother is an important contributory factor [6], it is possible that disturbances of non-esterified fatty acids [7], 3-hydroxybutyrate [8] and amino acids [9] may also be significant factors, particularly since some of these metabolites correlate better with such characteristic changes in the infant as increased birth weight [10, 11] than does maternal hyperglycaemia [12].

These gestational diabetics are frequently treated initially by diet [13], using calorie and carbohydrate restriction and this is often sufficient to control blood glucose levels. We were interested in the effect of dietary treatment on other metabolites which might influence fetal development and have therefore measured the levels of blood glucose and other major intermediary metabolites over 24 h during late pregnancy in gestational diabetics before and after treatment with a 150-g carbohydrate diet. The results were compared with similar measurements in a group of normal non-diabetic women of the same gestational age.

Subjects and Methods

Subjects

Three groups of women were studied (Table 1) who were of similar age, weight, percentage desirable body weight, parity and gestational age at the time of study. Group A consisted of seven women with normal oral glucose tolerance in the third trimester. Their pregnancies were uncomplicated apart from suspected placenta praevia without bleeding in three cases, mild hypertension resolving on bed rest in one, and one woman with fetal growth retardation in a previous pregnancy.

Two groups of women with gestational diabetes were studied. The first diabetic group (group B) included seven women referred from the antenatal clinic with suspected gestational diabetes and studied at the time of diagnosis prior to any dietary treatment. The second group (group C) included seven women studied after treatment with a 150-g carbohydrate-restricted diet. Four of the gestational diabetic women were studied before and after dietary treatment and are included in both groups B and C. Patients in all groups had previously normal obstetric histories. Patients in groups B and C had normal glucose tolerance 6 weeks postpartum. In all patients studied, informed consent was obtained and the work had Ethical Committee approval.

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Table 1. Clinical details of the three groups of women studied

	No. of patients studied	Age (years)	Weight (kg)	% Desirable body weight[a]	Parity (infants/ group)	Gestational age (weeks)
Group A	7	28 ± 2.0	65 ± 9.4	110 ± 19.3	4	34 ± 2.2
Group B	7	27 ± 7.8	68 ± 13.7	112 ± 17.8	6	32 ± 5.0
Group C	7	27 ± 6.9	67 ± 13.6	114 ± 18.4	4	34 ± 4.2

Results expressed as mean ± SD
[a] Percent of desirable weight was calculated using pre-pregnant weights from the tables of the Metropolitan Life Insurance Society [29]
Group A: non-diabetic women; *group B:* gestational diabetics before treatment; *group C:* gestational diabetics controlled on a 150 g carbohydrate diet

Table 2. Blood glucose, lactate, alanine and total ketone body levels during 24 h in the three groups of women studied

	Blood glucose (mmol/l)	Blood lactate (mmol/l)	Blood alanine (mmol/l)	Blood total ketone bodies (mmol/l)
Group A	4.65 ± 0.82	1.05 ± 0.18	0.31 ± 0.03	0.11 ± 0.04
Group B	5.35 ± 3.06	1.14 ± 0.42	0.31 ± 0.10	0.19 ± 0.05[b]
Group C	5.40 ± 1.74	0.78 ± 0.22[a]	0.27 ± 0.07	0.26 ± 0.15

Results expressed as mean ± SD. Groups as in Table 1
[a] $p < 0.10$ compared with group B
[b] $p < 0.01$ compared with group A

Definitions

The diagnosis of gestational diabetes was based on the recommendations of the British Diabetic Association [14] and of Hadden [15], using a standard 50-g oral glucose tolerance test with venous blood samples. Tests were regarded as abnormal if either the fasting blood glucose or the 2-h blood glucose level was > 6.7 mmol/l. Only those gestational diabetics with normal fasting glucose levels and raised 2-h glucose levels were included in this study. The mean glucose levels for each group at 0 and 120 min after oral glucose were for group A: 3.8 ± 0.6 and 4.7 ± 1.1 mmol/l; group B: 4.6 ± 1.3 and 7.9 ± 0.7 mmol/l, group C: 5.0 ± 0.9 and 8.1 ± 0.5 mmol/l.

Protocol

Women in groups A and B did not restrict their diets before or during the study and each woman had an oral glucose tolerance test performed within 1 week of the study. Women in group C were on a 150 g carbohydrate diet at home before the study. The diet was given as 30 g at breakfast, 40 g at lunch and supper and 10 g at each snack time at mid-morning, mid-afternoon and before bed. The remaining 10 g was taken as milk throughout the day. Dietary advice was given and regularly reinforced by a trained dietitian and maintained on the ward during the metabolic profile. Whilst direct control of dietary intake as an outpatient was not possible, compliance in these pregnant diabetic subjects was thought to be good. The mean calorie intake of 2200 kcal (range approximately 1,900–2,600 kcal) was maintained at the pre-treatment level as carbohydrate intake was controlled.

A 24-h metabolic profile was obtained in each of the women who were admitted overnight prior to the study. The women were permitted to be ambulant during the study other than for 5 min rest prior to sampling. Samples were obtained from an indwelling cannula kept patent with normal saline, and blood was taken hourly from 0800 to 2400 h and at 0200, 0400, 0600 and 0700 h.

Metabolite Estimations

Two millilitres of blood was taken from the patients at each time point during the metabolic profile and added to cooled tubes containing 5% w/v perchloric acid. Precise amounts of blood and perchloric acid were determined gravimetrically. The tubes were then centrifuged twice and the supernatant separated within 10 min, being stored at −20 °C. Blood glucose [16], lactate [17], alanine [18], 3-hydroxybutyrate [19] and acetoacetate levels [20] were estimated using spectrophotometric techniques. Acetoacetate levels were measured within 24 h and other assays within 2 weeks. 3-hydroxybutyrate and acetoacetate levels at each sample time have been combined and expressed as total ketone bodies.

Statistical comparisons between groups have been made using Student's 't' test (Welch approximation) [21].

Results

The mean 24-h levels for glucose, lactate, alanine and total ketone bodies are shown in Table 2 and the variations of these metabolites during the 24-h period are shown in Figure 1. The diabetics tended to have higher glucose levels than non-diabetic women, although the differences were not significant. There was slightly more variation in blood glucose concentration during the day in the diabetics compared with the non-diabetic women, as assessed by comparison of the standard deviation of the means (group B – mean glucose SD = 1.20, range 0.75 to 3.44; group A – mean glucose SD = 0.80, range 0.62 to 0.97, but dietary treatment did not improve the variation in blood glu-

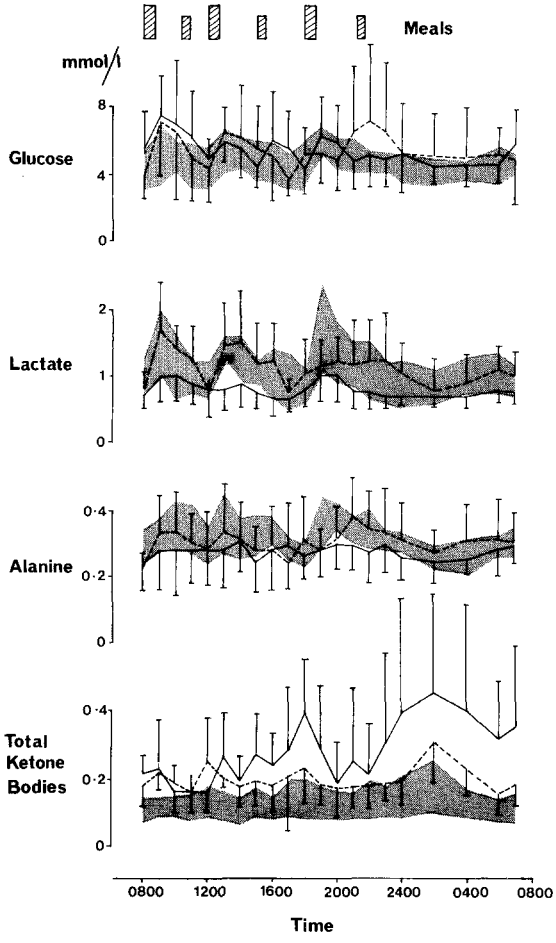

Fig. 1. Mean levels of blood glucose, lactate, alanine and total ketone bodies during the 24-h metabolic profiles in the third trimester of pregnancy in seven non-diabetic women (group A, shaded areas), seven gestational diabetics at diagnosis before dietary treatment (group B, broken lines and bars) and seven gestational diabetics during treatment with a 150-g carbohydrate restricted diet (group C, solid lines and bars). Shaded areas and lines and bars represent mean levels ± SD. Times of meals and snacks are represented by large and small hatched blocks respectively

cose during the day in the diabetics; group C – mean glucose SD = 1.25; range 0.62 to 2.97). Glucose levels tended to rise higher and remain elevated longer after meals in the diabetic group compared with the non-diabetic women, whereas the fasting glucose levels were not significantly different in each of the groups.

The lactate and alanine levels were similar in the non-diabetic women and the diabetics before treatment. Dietary restriction was associated with a fall in lactate levels ($0.05 < p < 0.10$ compared with untreated gestational diabetics). There was no significant reduction in alanine levels with dietary restriction. The reduction in mean 24-h lactate levels associated with dieting appeared to be related to the suppression of post-prandial rises of lactate.

Total ketone body levels were significantly elevated in the untreated diabetics compared with non-diabetic women ($p < 0.01$) and rose further in association with dietary treatment, although this did not reach significance possibly because of the greater variation in 24-h mean levels on diet (range 0.08 to 0.58 mmol/l). Total ketone body levels in untreated diabetics were persistently raised throughout the day with peaks occurring at the normal times before meals and overnight. Dietary treatment was associated with a further increase in ketone body levels during the afternoon, evening and overnight.

Discussion

The gestational diabetics in this study had minor abnormalities in carbohydrate metabolism demonstrated by the oral glucose tolerance test (White Class A1) and these were also apparent during the 24-h profiles, with a more pronounced and prolonged elevation of blood glucose after meals than in non-diabetic women. Although dietary treatment was not associated with significantly lower glucose levels, the patients were studied 2 weeks later in gestation, and since glucose tolerance is progressively impaired during pregnancy [22], dietary treatment could have prevented further deterioration of glucose homeostasis.

Increased levels of blood lactate normally occur after meals, due to increased glycolysis [23]. In the untreated gestational diabetics, marked increases in lactate occurred after meals paralleling closely the changes in blood glucose, which suggested that these women were able to secrete sufficient insulin to stimulate normal glycolysis. In the untreated gestational diabetics mean levels of blood lactate and alanine were very similar to those seen in non-diabetic women throughout the 24 h. On dietary treatment there was a reduction in mean blood lactate over the 24 h and this was most marked post-prandially, which may indicate that carbohydrate intake had been effectively limited so that there was reduced glycolysis. Alanine levels showed a general reduction compared with non-diabetic women throughout the day but similar levels during the night. It is possible that during the day, with limited carbohydrate intake, these women maintained a higher level of gluconeogenesis than the non-diabetic women and hence utilised more alanine and lactate. However, during the night absence of carbohydrate would have occurred in both groups with the same resultant degree of gluconeogenesis.

Increased lipolysis and ketone body production after extended fasting in normal pregnancy has been termed accelerated starvation [24]. It might be expect-

ed that diabetic patients would be more susceptible to this and indeed raised profiles of non-esterified fatty acids [25] and of fasting blood ketone bodies [26] have been observed in gestational diabetics. The persistently raised levels of ketone bodies in the untreated diabetics in this study throughout the day with peaks before meals and overnight is in keeping with accelerated starvation. It would seem unlikely to be due to relative insulin lack since carbohydrate restriction exacerbated the ketone body changes which were still more pronounced before meals and throughout the night. Whilst dietary treatment did not significantly increase mean ketone body levels, there was a marked trend towards this as well as to an increased variability of ketone body production throughout the day.

Metabolic factors other than blood glucose are known to be important in fetal development. Certain amino acids for instance have been shown to be potent stimuli to pancreatic insulin secretion [27] and may be a primary cause of fetal B cell hyperplasia characteristically seen in large infants of diabetic mothers. It has also been shown that small changes of non-esterified fatty acid and ketone levels can have marked effects on placental metabolism and fat deposition in vitro and these changes may be implicated in the in vivo changes in placental structure and function in diabetic mothers [8]. Clinically an association between maternal diabetes complicated by acetonuria and neuropsychological deficits in the children has been suggested to be present in both severe diabetes as well as in mild, including gestational diabetes [28], though other confirmatory studies are required. These results would seem to indicate that metabolic disturbances do occur in the presence of near normal glucose homeostasis even in the mildest gestational diabetics, which are exacerbated by dietary treatment and which could possibly influence fetal development. In treating gestational diabetics therefore, as in established diabetics, it is essential to obtain the best overall metabolic control. Treatment regimes however, which worsen some of the metabolic abnormalities of gestational diabetics would seem undesirable and carbohydrate restriction alone inappropriate. It is possible that insulin treatment with less severe dietary restriction may be a more rational approach to the management of these patients.

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