CONDENSED REPORT

Diurnal plasma free fatty acid profiles in normal and diabetic pregnancies

M D G GILLMER, R W BEARD, N W OAKLEY, FIONA M BROOKE, M C ELPHICK, D HULL

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Summary

Diurnal plasma free fatty acid (FFA) and glucose concentrations were measured in 23 normal women, 13 chemical diabetics, and 11 insulin-dependent diabetics in late pregnancy. The mean diurnal FFA value was lowest in the insulin-requiring diabetics and highest in the chemical diabetics. Although no association was found between percentile birthweight and maternal mean fasting or diurnal FFA, there was a positive correlation between percentile birthweight and both mean maternal fasting plasma glucose and mean diurnal glucose in normal and chemical diabetic women.

These observations support the view that glucose is the major substrate used by the fetus, but birthweight may be influenced more by the total substrate crossing the placenta than by maternal levels of either glucose or FFA alone.

Introduction

Infants born to diabetic mothers are heavier than those of normal women and have an increased adipose tissue mass.[1-3] This is generally considered to result from the transplacental transfer of excessive amounts of glucose, resulting in fetal hyperinsulinaemia and accelerated triglyceride synthesis.[4] Szabo and Szabo[5] have questioned this concept, arguing that glucose is not the major precursor of fetal triglyceride fatty acids but only of the α-glycerophosphate necessary for triglyceride formation. They postulated that the higher plasma free fatty acid (FFA) concentration in pregnant diabetics[6 7] results in increased transfer of FFA to the fetus.

We measured the diurnal plasma FFA and glucose concentrations in normal women and women with chemical and insulin-dependent diabetes during the last trimester of pregnancy. To assess the possible contribution of maternal plasma glucose and FFA to the fetal adipose mass we analysed the relation between diurnal and fasting plasma FFA and glucose concentrations in individual patients and the precentile birthweights of their infants.

The diurnal plasma insulin concentrations in the normal and chemical diabetic women were also measured and these were correlated with the corresponding diurnal plasma glucose and FFA levels in an attempt to gain insight into the homoeostatic mechanisms that control the circulating concentrations of these metabolites.

Patients and methods

Fifty women were studied late in pregnancy. Thirteen had severe diabetes and had been on insulin treatment for six to 26 years. The remaining patients comprised 31 women with one or more of the features of potential diabetes[8] and six controls.

The insulin-dependent patients were studied after at least two weeks in hospital, when optimal diabetic control had been achieved with careful dietary management and twice-daily injections of soluble and isophane insulin. They were studied on their usual treatment regimen. The remaining women were admitted to hospital and the study started at 1000. Blood samples were collected hourly until 2000, and during this time the patient was encouraged to be active. All but the severe diabetics were given a total daily carbohydrate intake of 180 g, of which 40 g was consumed at home in the form of breakfast. Main meals containing 40 g carbohydrate were taken at 1200 and 1800, and snacks containing 20 g carbohydrate were taken at 1100, 1500, and 2100. From 2200 until 0800 the next morning blood samples were taken every two hours. After the overnight fast a five-hour 50-g oral glucose tolerance test (GTT) was performed on the non-insulin requiring patients. This was started at 0900 and during the test the patient remained awake and semi-recumbent in bed and was not allowed to drink or smoke.

Patients were assigned to a normal or a chemical diabetic group on the basis of the GTT result, the response to this test being assessed in terms of the area under the three-hour glucose curve.[9] In non-pregnant patients a glucose area of 800 units correlates closely with other widely used criteria of abnormal glucose tolerance and is taken to indicate chemical diabetes. But in view of the decline in fasting plasma glucose that occurs during pregnancy[10 12] the upper limit of normal for the third trimester was reduced to 750 units. Twenty-four of the patients studied were classified as normal and 13 as chemical diabetics. There was no difference between the results of the 18 potential diabetics with normal GTT values and the six controls, and these two groups were therefore combined.

In calculating the mean data and performing between-group comparisons three of the patients were omitted: one normal woman with a twin pregnancy and two insulin-dependent diabetic patients who delivered congenitally malformed infants. The three groups of patients were well matched for age, weight, height, and gestation at study (table I).

The study was performed between November 1972 and July 1974. It was originally designed to examine diurnal carbohydrate metabolism in normal and diabetic women in pregnancy. But when Szabo and Szabo published their hypothesis in 1974[5] we decided to measure the FFA concentrations in the stored plasma, and this was done in November 1974. The blood samples had been collected through an indwelling cannula in an antecubital vein. This was kept patent by flushing with 1 ml of heparin saline (10 units sodium heparin/ml) after each blood sample had been collected. The residual heparinised saline (0·5 ml) was aspirated from the cannula immediately before the next sample was taken. Intravenous injection of heparin stimulates

Department of Obstetrics and Gynaecology, St Mary's Hospital Medical School, London W2
M D G GILLMER, MB, MRCOG, lecturer
R W BEARD, MD, FRCOG, professor

Department of Human Metabolism and Alexander Simpson Laboratory, St Mary's Hospital Medical School, London W2
N W OAKLEY, MD, MRCP, senior lecturer
FIONA M BROOKE, BSC, PHD, research assistant

Department of Child Health, University Hospital and Medical School, Nottingham NG7 2UH
M C ELPHICK, BSC, PHD, biochemist
D HULL, FRCP, DCH, professor

TABLE I—*Details of the 47 patients studied. Values are means ± SD*

	No of patients	Age (years)	Weight at delivery (kg)	Height (cm)	Gestation at study (weeks)	Gestation at delivery (weeks)	Birthweight (kg)	Birthweight (percentile)
Normal subjects	23	26·3 ± 4·7	72·71 ± 15·64	159·26 ± 5·35	33·9 ± 2·2	33·8 ± 1·28	3·215 ± 0·485	44·91 ± 23·77
Chemical diabetics	13	27·5 ± 6·1	73·59 ± 9·77	161·5 ± 5·70	33·2 ± 2·9	38·5 ± 1·13	3·387 ± 0·407	59·38 ± 22·78
Insulin-dependent diabetics	11	25·5 ± 3·9	70·98 ± 7·41	162·91 ± 6·46	34·7 ± 1·2	37·6 ± 0·51	3·327 ± 0·466	60·91 ± 26·67

TABLE II—*Mean diurnal plasma glucose and FFA concentrations (± SD) in three groups of patients studied*

	No of patients	Fasting plasma glucose (0800 h) (mmol l)	Mean diurnal plasma glucose (mmol l)	Fasting plasma FFA (0800 h) (mmol/l)	Mean diurnal plasma FFA (mmol/l)
Normal subjects	23	4·05 ± 0·52	4·70 ± 0·38	0·65 ± 0·26	0·68 ± 0·20
Chemical diabetics	13	4·67 ± 0·82	5·61 ± 1·03	0·79 ± 0·46	0·77 ± 0·34
Insulin-dependent diabetics	11	5·24 ± 1·87	6·02 ± 1·26	0·51 ± 0·16	0·45 ± 0·11

Conversion: SI to traditional units—Glucose: 1 mmol l ≈ 18 mg 100 ml.

lipoprotein lipase activity and so raises plasma FFA concentrations,[13] an effect which is more pronounced in pregnancy.[14] But the dose of heparin usually used is about 1000 times that used to maintain cannula patency in this study, and all subjects were investigated using an identical blood sampling technique, so it is unlikely that our findings could have been distorted by a heparin artefact.

Blood was collected into lithium heparin tubes, centrifuged, and the plasma separated immediately. Glucose was measured by an automated glucose oxidase-peroxidase method[15] within 24 hours of collection on plasma kept at 4°C. FFA concentration was measured by an automated modification of Duncombe's method[16] and insulin concentration by radioimmunoassay using a charcoal separation technique.[17] The plasma was stored at −20°C but the samples of the normal and chemical diabetic women had been thawed once for plasma insulin determination.

The means of all the plasma glucose and FFA values obtained from the start of the study at 1000 until its conclusion at 0800 the next morning were calculated as the mean diurnal values. To assess the insulin response to glucose the area under the insulin curve from 1000 to 0800 the next morning was calculated as the diurnal insulin area. The mean weight of all infants immediately after birth is shown in table I. The individual birthweights were corrected for differing gestational age and sex and expressed as percentiles.[18]

Statistical analyses were performed using the SPSS package available at the University of London Computer Centre. Student's *t* test was used to assess the significance of the difference between the means of the study groups, and Pearson's correlation coefficient was used to assess correlation.

Results and comment

The mean plasma glucose and FFA concentrations during the diurnal study and five-hour GTT in normal women and chemical diabetic patients are shown in fig 1 and those for the diurnal study on insulin-dependent diabetics in fig 2. Mean fasting and diurnal glucose and FFA values are shown in table II. The plasma glucose and insulin concentrations have been reported,[19] but the glucose results are summarised as they are relevant to the ensuing discussion.

PLASMA FFA RESULTS

Throughout the diurnal study the mean plasma FFA concentrations of the women with chemical diabetes were higher than those of the normal women, but at no point did this difference reach statistical significance, nor was there any difference between the mean diurnal values of the two groups. In contrast, the mean plasma FFA concentrations of the insulin-treated women were lower than those of the normal group for most of the study and this was reflected in a highly significant difference between the mean diurnal FFA values of these two groups ($P < 0{\cdot}001$).

The marginally raised plasma FFA concentrations in many patients at the start of the study probably resulted from anxiety at the time of introducing the intravenous cannula. A similar effect was noted in a longitudinal study of fasting FFA levels during pregnancy,[20] in which changes of as much as half the mean value occurred with samples taken a few minutes apart, even when the stress of venepuncture was minimised by the use of an indwelling cannula. In general, the expected fall in FFA concentration after meals, followed

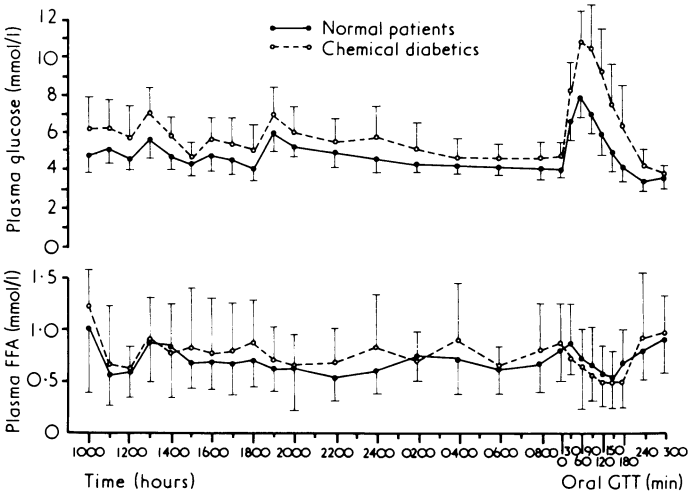

FIG 1—Mean plasma FFA and glucose concentrations during diurnal study and oral GTT in 23 normal women and 13 chemical diabetics.
Conversion: SI to traditional units—Glucose: 1 mmol/l ≈ 18 mg/100 ml.

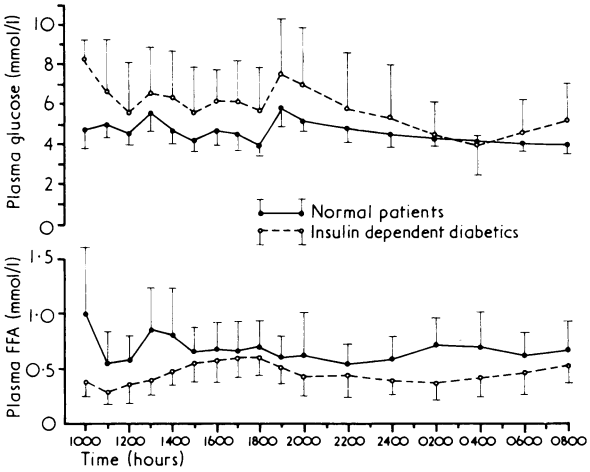

FIG 2—Mean plasma FFA and glucose concentrations during diurnal study in 23 normal women and 11 insulin-dependent diabetics.

TABLE III—*Correlation coefficient (r) between percentile birthweight and maternal plasma glucose and FFA concentrations*

	Fasting plasma glucose	Mean diurnal plasma glucose	Fasting plasma FFA	Mean diurnal plasma FFA
Percentile birthweight excluding children of insulin-treated mothers (n = 36)	0·347*	0·311‡	0·154	0·126
Percentile birthweight of whole group (n = 47)		0·241‡		0·063

*P < 0·02. ‡P < 0·05.

by a gradual rise with increased fasting, was observed in both the normal and chemical diabetic women. An unexpected, but significant, increase in FFA concentration was seen during the hour after lunch in both groups (P < 0·001). Stable or increasing FFA concentrations have been observed during the first hour after lunch in gestational diabetic and normal women in late pregnancy[21] and in male and female normal and diabetic subjects after the midday meal, but not after breakfast or supper.[22]

A rapid fall in plasma FFA concentrations after oral glucose was seen in all subjects tested. The insulin-dependent diabetics displayed a completely different diurnal FFA pattern from women in the normal and chemical diabetic groups. After an initial decline between 1000 and 1100 (P < 0·02) plasma FFA concentrations progressively increased until 1800 (P < 0·001). After the evening meal and insulin injection the concentration again declined and was lowest at 0200 (P < 0·001)—about two hours before the nadir in plasma glucose concentration. Subsequently the levels rose progressively until the conclusion of the diurnal study at 0800 (P < 0·001).

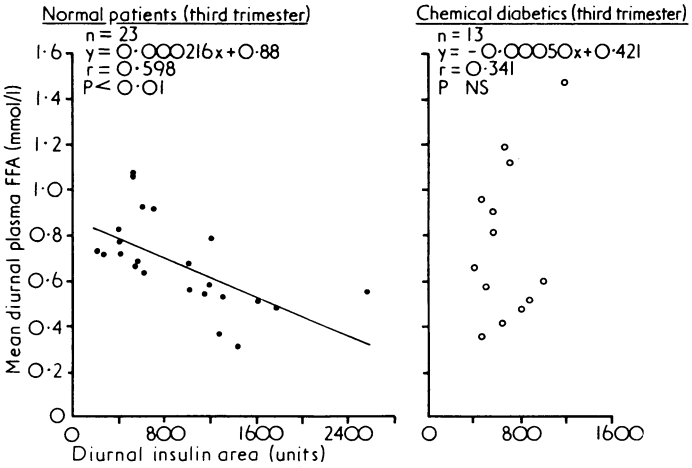

FIG 4—Correlation between mean diurnal plasma FFA value and diurnal insulin area in 23 normal women and 13 chemical diabetics.

PLASMA GLUCOSE RESULTS

Fasting plasma glucose concentrations were within normal limits in most gestational diabetics studied, reflecting the relatively mild degree of carbohydrate intolerance in these women. This selection was dictated by the protocol requirement that women should remain untreated during the pregnancy so that observations made early in the third trimester of pregnancy might truly reflect the degree of carbohydrate intolerance prevailing during the later weeks of pregnancy. Only three of the 13 chemical diabetics had fasting plasma glucose values outside the normal range, at 5·61, 5·72, and 6·33 mmol/l (101, 103, 114 mg/100 ml). Table III, in which metabolic measurements have been related to percentile birthweight, includes an analysis from which insulin-treated women have been excluded because the fasting plasma glucose concentration of insulin-treated women has no physiological significance, and their exclusion makes our patients comparable with those studied by Szabo *et al.*[23]

The overall mean diurnal glucose values were significantly higher for the chemical diabetics than for the normal women, which indicated that mild carbohydrate intolerance detected by GTT was closely reflected in the plasma glucose pattern obtaining during a normal day. Intensive hospital treatment with insulin did not bring the overall mean diurnal plasma glucose concentration of the severe diabetics down even to the level found in the chemical diabetic group.

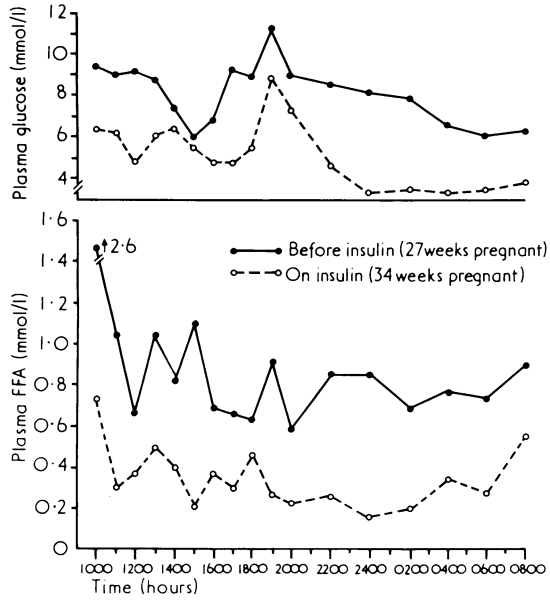

FIG 3—Plasma FFA and glucose concentrations in one patient before insulin treatment, at 27 weeks of pregnancy, and after seven weeks on insulin treatment, at 34 weeks of pregnancy.

EFFECT OF INSULIN ON FFA CONCENTRATIONS

To investigate the lower diurnal FFA concentrations in the insulin-treated diabetics, glucose and FFA profiles were studied in a patient with chemical diabetes before insulin treatment at 27 weeks and after seven weeks on insulin (fig 3). Insulin was given at the same times as in the insulin-dependent diabetic group. A sharp decline in the mean plasma glucose value (±SD) from 8·19±1·44 mmol/l (148±26 mg/100 ml) to 5·25±1·55 mmol/l (95±28 mg/100 ml) was associated with a fall in mean FFA value from 0·91±0·46 mmol/l to 0·36±0·15 mmol/l (P < 0·001).

To assess the relation between endogenous insulin production and plasma FFA concentration the area under the diurnal insulin curve was calculated for each of the normal and chemical diabetic women. In normal women there was a significant negative correlation between the mean diurnal plasma FFA and the diurnal insulin area (r = −0·598; P < 0·01), but this was not present in the chemical diabetic group (r = 0·341) (fig 4). There was a negative correlation between the mean diurnal plasma glucose and FFA values in the normal (r = −0·483; P < 0·05) but not the chemical diabetic women (r = 0·338). A significant positive correlation was found between the mean diurnal plasma glucose and insulin areas in 23 normal women (r = 0·577; P < 0·01) but not in the 13 chemical diabetics (r = 0·053).

MATERNAL PLASMA GLUCOSE AND FFA CONCENTRATIONS AND INFANT BIRTHWEIGHT

The mean percentile birthweights of the three groups studied are shown in table I. The infants of the chemical and insulin-dependent

diabetic patients were of similar birthweight, while the mean weight of the infants of the normal women was lower but not significantly so.

The correlations between the percentile birthweight and the maternal plasma glucose and free fatty acid concentrations are shown in table III and figs *a* and *b*.* Fasting and mean diurnal plasma glucose concentrations both showed a significant positive correlation with percentile birthweight in normal women and those with chemical diabetes, but no correlation was shown between plasma FFA concentration and percentile birthweight.

The correlation between mean diurnal plasma glucose and percentile birthweight was reduced when the 11 insulin-dependent diabetics were included ($r = 0.241$) but remained significant ($P = 0.05$). There was, however, no correlation between mean diurnal plasma FFA and percentile birthweight in the whole group.

Discussion

Szabo and Szabo have suggested that fetal storage triglyceride is mainly derived from circulating plasma FFA.[5] They supported this hypothesis by showing that maternal plasma FFA is positively correlated with birthweight.[23] This finding, they argued, is in keeping with the acknowledged fact that plasma FFA concentrations are higher in untreated diabetics, who also have heavier babies than normal. Their views were supported by the demonstration in various species[21-29] of placental transfer of FFA from mother to fetus in vitro[30 31] and in vivo.[32-34] The dependence of this transfer on the existence of a maternal-fetal gradient is suggested by the observation that there is an umbilical vein-artery FFA concentration difference of about 0·06 mmol/l, which is positively correlated with the maternal FFA concentration at delivery.[32 33] Against the Szabos' hypothesis is the finding that the FFA concentration in the umbilical blood of the babies of normal and diabetic women is not significantly different.[33 35] This last observation might be explained by the known effect of insulin in depressing plasma FFA, and hence it is preferable to exclude insulin-treated diabetics when correlating maternal FFA concentrations with birthweight.

Our findings do not support the Szabo hypothesis. Firstly, we observed that the birthweights of infants of women with chemical and insulin-dependent diabetes were almost identical despite a wide difference in plasma FFA concentrations. Secondly, we could not find any correlation between maternal FFA levels and percentile birthweight, even when the insulin-treated diabetics were excluded from the comparison. Our results are more convincingly explained by the original proposal of Pedersen[1] that glucose derived from the mother is the major precursor of fetal storage triglyceride. The tendency in our study for the percentile birthweights of the infants of both the chemical and insulin-treated diabetic women to be heavier than those of the normal women is more likely to be explained by the higher mean diurnal and fasting glucose concentrations of their mothers.

The extent to which the fetus uses the various nutrients that are available to it is difficult to determine. The substrate environment of the fetuses in the three groups we studied differs considerably. The reduced plasma FFA concentration of the insulin-treated diabetics may be ascribed to the effect of exogenous insulin, which by inhibiting lipolysis and enhancing lipogenesis overcomes that state of accelerated starvation characteristic of late pregnancy.[36] The observation that the diurnal plasma glucose concentration tends to be raised despite a depressed FFA concentration can be explained by the differential action of exogenous insulin, which inhibits lipolysis but fails to achieve normoglycaemia, a concept which is supported by the earlier studies of Burt on normal women in late pregnancy.[37] In the untreated mothers with chemical diabetes the raised glucose and FFA concentrations can be ascribed to insulin deficiency. Possibly the higher plasma glucose concentration presented to the fetus of the insulin-dependent mother is offset by the lower FFA concentration. Fetal size may thus be influenced more by total substrate crossing the placenta than by maternal concentrations of either glucose or FFA alone.

*Copies of figs *a* and *b* may be obtained from the authors.

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Copies of the unpublished tables may be obtained from Dr M D G Gillmer, Department of Obstetrics and Gynaecology, St Mary's Hospital Medical School, London W2 1PG.

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