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CARBOHYDRATE METABOLISM IN PRE-ECLAMPSIA

BY

M. M. SINGH
Lecturer in Human Reproduction and Obstetrics
University of Southampton

Summary

Intravenous glucose tolerance tests were performed on 30 primigravidae with pre-eclampsia and 15 normal primigravidae in late pregnancy. The groups were matched for age, height and weight and had no stigmata of potential diabetes. Plasma glucose, plasma immunoreactive insulin and plasma placental lactogen (HPL) levels were measured before (fasting) and at timed intervals after the glucose challenge; the glucose response was expressed as the increment index. The patients with severe pre-eclampsia had significantly lower fasting plasma glucose levels than those with mild pre-eclampsia and normal pregnancies. The mean increment index in both the severe and mild pre-eclamptic groups was significantly lower than that of the normal pregnant group. Fasting HPL levels were significantly lower in patients with severe pre-eclampsia than in those who had mild pre-eclampsia or a normal pregnancy. Both the fasting plasma insulin and insulin response following glucose injection were lower in patients with severe pre-eclampsia than in those with mild pre-eclampsia or a normal pregnancy. The differences however were not statistically significant. The results of this study suggest that carbohydrate metabolism in severe pre-eclampsia is altered to an extent similar to that in patients with chemical gestational diabetes, and this alteration may be due to maternal β-cell anoxia caused by the vascular changes in pre-eclampsia.

THE literature, although scanty and inconclusive, does suggest that there is some alteration of carbohydrate metabolism in pre-eclampsia, but the nature of this change is uncertain and the suggested aetiological factors are debatable.

Although it is clear from the reports of Stander and Harrison (1929) that there is hyperglycaemia before, during and after eclamptic convulsions, controversy exists concerning blood glucose levels in pre-eclamptic patients. Titus *et al* (1930) claim that hypoglycaemic levels are a predominant and fairly constant feature of true pre-eclampsia.

Burt (1955) in his study of 13 pre-eclamptic patients showed a relative hyperglycaemic response following 25 g of intravenous glucose compared with that of normal pregnant subjects. Yamamoto (1967), reporting the results of oral glucose tolerance tests in nine patients with mild and three with severe pre-eclampsia, found that in pre-eclampsia of late pregnancy the blood sugar values 30 minutes after glucose loading was slightly higher than those in subjects in late normal pregnancy.

Madsen *et al* (1973), using the oral glucose tolerance test in non-pregnant patients, normal pregnancy and patients with 'slight' pre-eclampsia, reported no differences when the areas below the glucose curves were compared in the three groups.

From these reports, it appears that glucose homeostasis in pre-eclampsia is impaired but the precise nature of this impairment is uncertain.

Govan *et al* (1951) reported that exogenous insulin produced an attenuated glucose response in toxaemic patients compared with normal pregnant women. Similar findings were reported by Burt (1957). Govan *et al* (1951) thought their results might indicate pituitary overactivity, but Burt (1957) suggested that they were due to corticosteroid influences.

Reports of plasma insulin levels in pre-

eclampsia both in the fasting state and after glucose loading are conflicting. Yamamoto (1967) reported that plasma insulin values following a 50 g oral glucose load were markedly depressed in both mild and severe pre-eclampsia; Madsen *et al* (1973) found that the insulin response was greater in these patients.

The aim of this study was to determine the nature of the disturbance of glucose homeostasis in pre-eclampsia and to assess the factors responsible for the disturbance.

Methods

Selection of patients

All patients were aged between 16 and 28 years and did not have a family history of diabetes, significant obesity, fasting glycosuria or polyhydramnios. The gestational age was 34 to 38 weeks by sure dates.

The study group consisted of patients with mild and severe pre-eclampsia. Pre-eclampsia was defined as a blood pressure of 140/90 mm Hg or over on two or more occasions separated by one day after the 26th week of gestation and the condition was considered to be mild when there was less than 0·25 g of protein per litre of urine and severe when the proteinuria was greater than 0·25 g per litre (Nelson, 1955). This study group was compared with a group of patients with normal pregnancies matched for age, weight and gestation.

Patients for the normal group were recruited from antenatal clinics and the pre-eclamptic group were patients admitted from antenatal clinics or from general practitioner units.

In order to avoid the possible association between resting and abnormal glucose tolerance, all patients in the pre-eclamptic group were tested within 24 hours of admission and after the stipulated overnight fast.

The groups were subdivided into categories for maternal weight gain between the 20th and 30th week of gestation according to the following criteria: low weight gain was taken as below 0·32 kg/week; normal weight gain as between 0·33 and 0·56 kg/week and high weight gain as greater than 0·56 kg/week.

Clinical methods

Patients for testing were fasted for at least 12 hours and after resting for approximately 30 minutes a teflon cannula was inserted into a vein, preferably in the antecubital fossa. A two-way stopcock was attached to the cannula to facilitate frequent sampling. After two fasting blood samples were taken, 50 ml of a 50 per cent dextrose solution was injected intravenously in the other arm over a period of three to four minutes.

Further blood samples were obtained at two-minute intervals for the first ten minutes and then at ten-minute intervals for the following 50 minutes, the test being completed after one hour.

The blood samples were collected in heparinized tubes and centrifuged. The plasma samples were divided into three aliquots and the plasma glucose estimated immediately, on the glucose auto-analyzer, using one aliquot. The remaining two aliquots were stored in a deep freeze for estimation of insulin and human placental lactogen (HPL).

Laboratory methods

Plasma glucose estimations using the auto-analyzer. Glucose estimations were carried out on plasma samples utilising the enzyme system glucose-oxidase/peroxidase on an auto-analyzer. This method is specific for glucose and other reducing substances, which might produce unduly high values, do not interfere. In this method, glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide and the peroxidase then catalyzes the transfer of oxygen to a chromogenic oxygen receptor. The analyzer measures the rate of consumption of oxygen which is directly proportional to the concentration of glucose in the sample.

Calculation of the increment index. The result of the intravenous glucose tolerance test (IVG TT) was expressed as an increment index as described by Duncan (1956) and represents the rate of fall of blood glucose as a percentage of the value in excess of the fasting level. Amatuzio *et al* (1953) reported that if the log of the increment of blood glucose is plotted against time, a straight line is obtained and the increment index can then be calculated using the formula $\frac{\log_e 2}{t}$ where t is the time in minutes required for the blood glucose increment value at any point to be halved. The line used for the calculation

of the increment index was the one that best fitted the increment plasma glucose at 4, 10, 20, 30, 40, 50 and 60 minutes, plotted on a semi-logarithmic scale. An increment index of 2·97 or less was regarded as abnormal and indicative of chemical diabetes.

HPL assay. HPL estimations were performed in the Department of Chemical Pathology at St Bartholomew's Hospital, London, with kind permission of Professors Landon and Chard. A radioimmunoassay method was employed as described by Letchworth *et al* (1971) with the exception that in this study the assay was carried out manually.

Insulin assay. Plasma immunoreactive insulin was measured in duplicate by a radioimmunoassay method incorporating the double antibody technique which closely resembles that described by Hales and Randle (1963) using the Insulin Immunoassay Kit as prepared by the Radiochemical Centre, Amersham.

RESULTS

Fasting plasma glucose levels

The fasting plasma glucose levels are given in Table I. The values represent the mean of two samples taken five minutes apart in the fasting state and before injection of glucose. The mean fasting plasma glucose level in the severe pre-eclamptic group of 60·0 mg/100 ml is significantly lower than the mean fasting levels in the mild and control groups: 66·2 and 65·8 mg/100 ml respectively ($p<0·01$).

Glucose tolerance tests

Table II shows the actual values of the increment indices. The mean values for both the severe and mild pre-eclamptic groups are significantly lower than the mean for the control group ($p<0·01$). However, when the mean values for the severe and mild groups are compared the difference is not significant.

In this study 10 out of 15 patients with severe pre-eclampsia had increment indices below 2·97 and were therefore considered to be abnormal (Table III). This is a significant finding compared with the number of abnormal tests in the mild pre-eclampsia and control groups.

TABLE I

Fasting plasma glucose in patients with pre-eclampsia and in normal pregnancy

Classification	No. of patients	Fasting plasma glucose (mg/100 ml) Mean ± SD
Severe pre-eclampsia	15	60·0 ± 6·23
Mild pre-eclampsia	15	66·2 ± 7·53
Normal pregnancy	15	65·8 ± 6·66

Severe *vs* mild and normal—$p<0·01$
(Statistical evaluation by *t* test)

TABLE II

Increment indices in pre-eclampsia and in normal pregnancy

Classification	No. of patients	Increment indices Mean ± SD
Severe pre-eclampsia	15	2·78 ± 0·69
Mild pre-eclampsia	15	3·22 ± 0·54
Normal pregnancy	15	4·17 ± 0·95

Severe *vs* normal—$p<0·01$
Severe *vs* mild —not significant
Mild *vs* normal —$p<0·01$
(Statistical evaluation by *t* test)

TABLE III

Incidence of abnormal glucose tolerance tests in pre-eclampsia and in normal pregnancy

Classification	No. of patients	No. with abnormal tests	Per cent
Severe pre-eclampsia	15	10	66·7
Mild pre-eclampsia	15	4	26·7
Normal pregnancy	15	1	6·6

Severe *vs* normal—$p<0·01$
Severe *vs* mild —$p<0·05$
Mild *vs* normal —not significant
All pre-eclamptics *vs* normal—$p<0·05$
(Statistical evaluation by χ^2 test)

Fasting HPL levels

The mean HPL levels of two fasting samples were lower in the severe pre-eclampsia group (Table IV) than in patients with mild pre-eclampsia or a normal pregnancy.

The difference between the values for patients with severe pre-eclampsia and those with a normal pregnancy is significant at the 1 per cent level whereas the difference between the severe and mild pre-eclampsia groups is just significant at the 5 per cent level. However, when the values

TABLE IV

Fasting human placental lactogen (HPL) in pre-eclampsia and in normal pregnancy

Classification	No. of patients	HPL (μg/ml) Mean ± SD	HPL range (μg/ml)
Severe pre-eclampsia	15	4·43 ± 1·33	2 to 7
Mild pre-eclampsia	15	5·85 ± 2·23	3 to 8·5
Normal pregnancy	15	6·79 ± 1·45	5·1 to 10

Severe *vs* normal—$p<0·01$
Severe *vs* mild —$p<0·05$
Mild *vs* normal —not significant

for the mild pre-eclampsia and normal pregnancy are compared, the difference is not significant.

Although the mean fasting and post-glucose injection HPL levels in the severe pre-eclampsia group were lower than in normal pregnancy or with mild pre-eclampsia, there were two patients in the severe pre-eclampsia group whose levels were consistently within the normal range and above the mean quoted for the normal group, the range for the severe group being 2 to 7 μg/ml.

Similarly, in the mild pre-eclamptic group three patients had fasting and post-glucose injection HPL levels above the normal mean, the range in the mild group being 3 to 8·5 μg/ml.

HPL levels during intravenous glucose

Blood samples were taken at two-minute intervals for the first 10 minutes after injection of glucose and at 10-minute intervals thereafter until one hour. Two important features were found (Fig. 1). First, the clear and persistent separation of the HPL values in the three groups throughout the test. Second, the initial fall in HPL levels which occurs within the first six minutes and then the gradual rise to pre-injection levels by the end of the first hour. However, despite the marked increase in plasma glucose levels following the intravenous glucose load there was very little variation in plasma HPL levels.

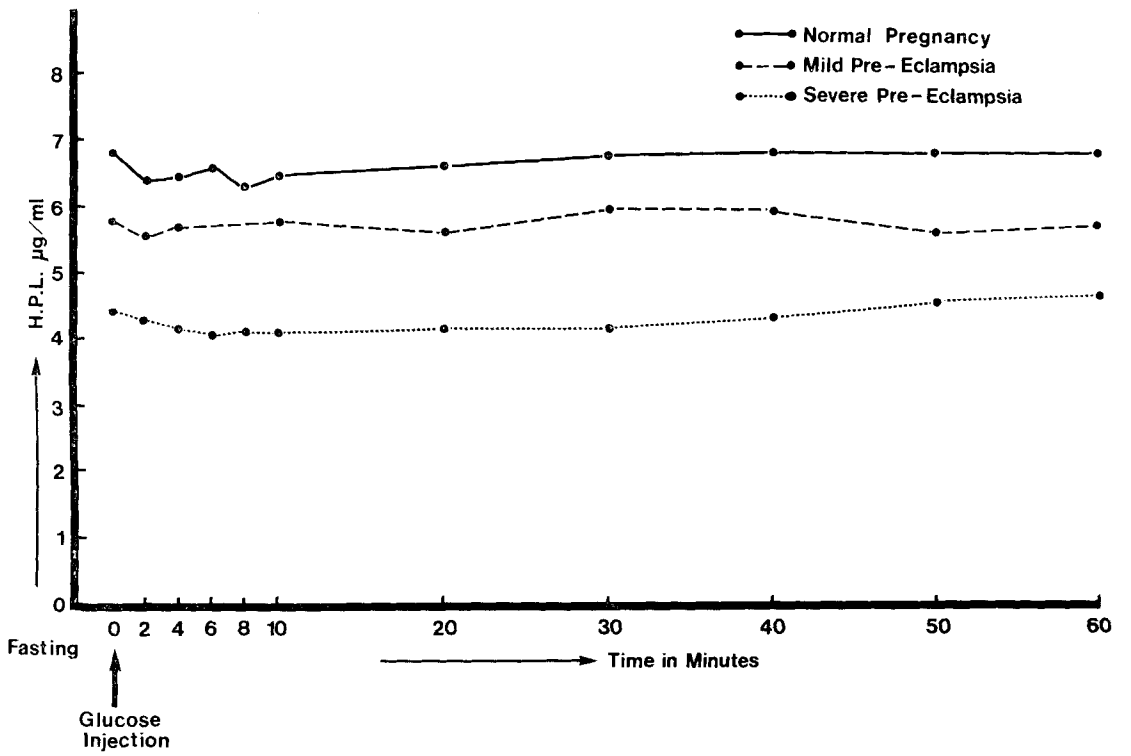

FIG. 1

Plasma human placental lactogen levels (HPL) during IVGTT

Fasting plasma insulin levels

Table V shows the levels of plasma insulin in the fasting state. The values represent the mean of two samples taken at least five minutes apart.

The patients in the severe pre-eclampsia group had a mean fasting plasma insulin level (18·73 μU/ml) which was lower than that in the mild pre-eclampsia patients (22·63 μU/ml) and normal control group (23·5 μU/ml). The differences, however, are not statistically significant, and there is marked individual variation.

TABLE V

Fasting plasma insulin in pre-eclampsia and in normal pregnancy

Classification	No. of patients	Insulin (μU/ml) Mean ± SD
Severe pre-eclampsia	15	18·73 ± 10·17
Mild pre-eclampsia	15	22·53 ± 10·24
Normal pregnancy	15	23·5 ± 9·0

Differences not statistically significant

Insulin response to glucose injection

Figure 2 shows the plasma insulin response following glucose injection in the three groups. Plasma immunoreactive insulin was measured at two-minute intervals for the first 10 minutes and at 10-minute intervals thereafter until one hour. Each point on the three graphs represents the mean values of the 15 patients in each group at the specified times.

It can be seen that the insulin response following glucose injection in the severe pre-eclamptics is lower when compared with the mild pre-eclampsia and with normal pregnancy. This separation between the groups persists until 40 minutes after glucose injection. The

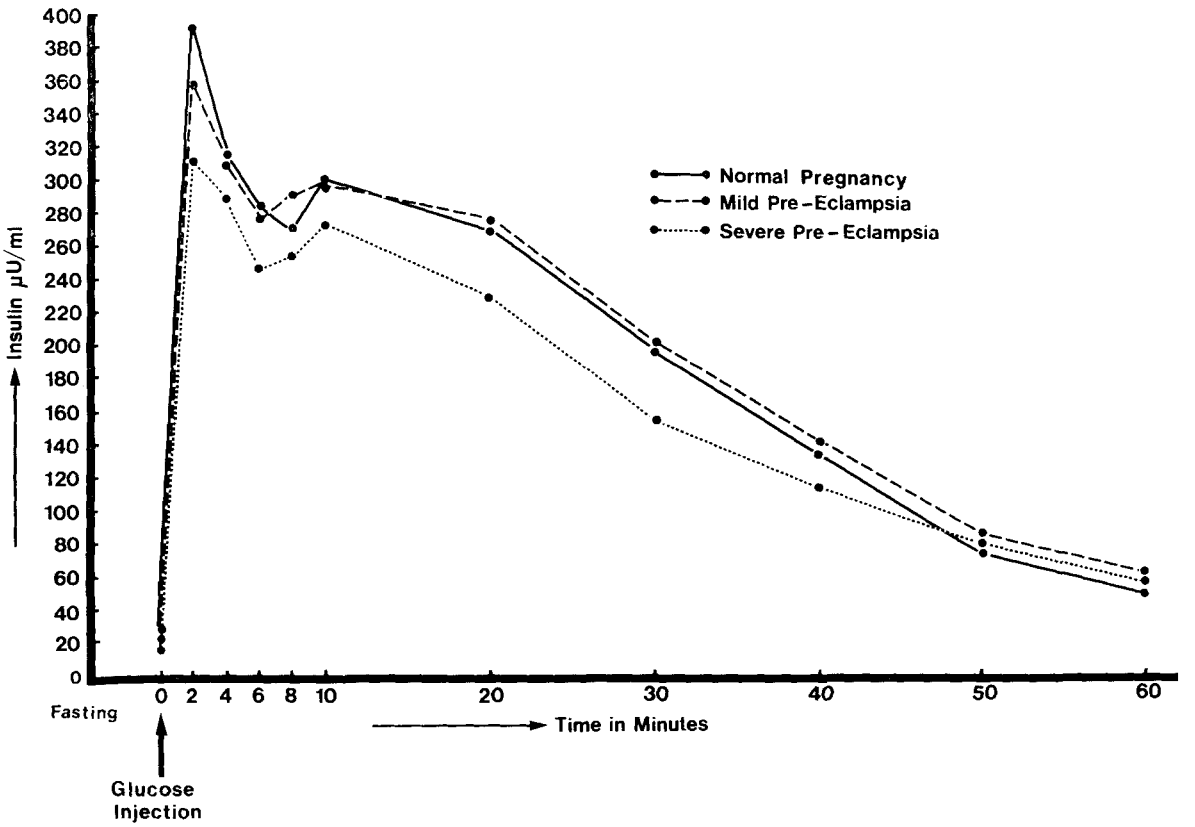

FIG. 2

Insulin response to glucose injection

values obtained for the mild pre-eclampsia patients lie much closer to the normal control group than to the severe pre-eclampsia group.

Discussion

As far as normal pregnancy is concerned reports suggest that the fasting blood sugar levels are lower than in non-pregnant women (Bleicher *et al*, 1964; O'Sullivan and Mahan, 1966; Trayner *et al*, 1967; Tyson *et al*, 1969). Reports of fasting blood sugar in late pregnancy range from 66 mg/100 ml to 71·8 mg/100 ml (Edstrom *et al*, 1974; Lind *et al*, 1973; Fisher *et al*, 1974).

The fasting plasma glucose levels reported in this study for mild pre-eclampsia and normal pregnancy (66·2 and 65·8 mg/100 ml respectively) are close to the values quoted by Edstrom *et al* (1974) and Lind *et al* (1973). The most significant observation, however, is that the mean fasting plasma glucose level in severe pre-eclampsia (60 mg/100 ml) was lower than the levels quoted above for normal pregnancy.

Although this observation of a lower fasting plasma glucose in pre-eclampsia is supported by that of Yamamoto (1967), Spellacy (1971) reported that blood glucose levels in pre-eclampsia were slightly higher than normal but he did not specify whether these were fasting or random values.

The reason for the significantly lower fasting plasma glucose in severe pre-eclampsia in this study is not clear. It could be that the liver in the patient with severe pre-eclampsia is compromised to such an extent that gluconeogenesis is impaired.

There were three reasons for the choice of the intravenous route for administration of glucose in this study. First, it was considered to be a more sensitive assessment of carbohydrate intolerance than the 50 g oral glucose challenge. Second, one of the aims of this study was to observe the functional response of the beta cell to a direct challenge and the intravenous route provided the most direct way of applying such a stimulus, and third, it was also intended for the intensive monitoring of plasma levels of glucose, HPL and insulin so that minor changes could be observed over short intervals.

In this study the rate of fall of the incremental plasma glucose (increment index) was used to interpret the result of the intravenous glucose tolerance test. This index is shown to be significantly lower for the patients with mild and severe pre-eclampsia than in the normal control group. Amatuzio *et al* (1953) suggest that an increment index of 2·97 or less is abnormal and indicative of chemical diabetes. If that standard is applied to this study, 10 out of 15 patients with severe pre-eclampsia and four patients with mild pre-eclampsia had abnormal increment indices. As none of these patients had any of the features associated with abnormal glucose tolerance tests, it can be concluded that the incidence of chemical gestational diabetes is greater in pre-eclampsia than in normal pregnancy.

As far as high weight gain and abnormal glucose tolerance are concerned, no direct relationship was demonstrated and this finding is supported by the observations made by Campbell (personal communication) who, in a study of 63 patients in the high weight gain category, reported five patients with abnormal responses to the 25 g intravenous glucose tolerance test; in four of these, however, the abnormality could have been due to other factors. The finding, therefore, of abnormal glucose responses in four out of five patients with severe pre-eclampsia and high weight gain reported here implies that the abnormal tolerance is related to the pre-eclampsia rather than the associated high weight gain.

The precise role of HPL in metabolic homeostasis has not yet been defined. Reports suggest that when it is given to normal persons or diabetics it impairs glucose tolerance (Beck and Daughaday, 1967; Samaan *et al*, 1968). It is claimed to have both insulinogenic and anti-insulin properties, and it has been suggested by Fioretti *et al* (1970) that one of the factors responsible for the greater stimuli to beta cell function is the elevated HPL levels in normal pregnancy. The present study provided a convenient model for investigation of the relationship of HPL to carbohydrate metabolism.

Previous data on the levels of HPL in relation to pre-eclampsia have been difficult to interpret because of the wide variation in results. The most extensive study was carried out by Spellacy *et al* (1971). In a study of 239 patients they showed

that HPL levels in pre-eclampsia were normal or low but not high. Lindberg *et al* (1973) in a study of 84 patients with mild and 14 patients with severe pre-eclampsia reported that patients with pre-eclampsia had lower mean plasma HPL levels than patients with a normal pregnancy, and that in patients with severe pre-eclampsia the mean values were usually lower than in those with mild pre-eclampsia. More recently Kelly *et al* (1975) have reported significantly lower HPL levels in severe pre-eclampsia than in normal pregnancy, although Letchworth and Chard (1972) have reported lower HPL levels in mild pre-eclampsia than in moderate and severe cases. The discrepancy between their results and those reported by other workers is probably due to the definition of pre-eclampsia adopted by them and the fact that the study was prospective, plasma samples being taken before the development of pre-eclampsia. In this study significantly lower levels of HPL were found in the severe pre-eclampsia patients compared with those with normal pregnancy. It is therefore unlikely that the abnormal glucose tolerance observed in the pre-eclamptic cases is related to HPL levels in this condition.

Insulin remains the key hormone in the control of carbohydrate metabolism and any observed alterations in glucose homeostasis must take it into account. Although plasma insulin results are always viewed with suspicion because of the different assay systems, the values reported in this study are to be interpreted only in comparing the groups studied and not as absolute values for comparison with other studies. In this study the mean fasting plasma insulin level in the severe pre-eclampsia group was lower than that in mild pre-eclampsia and in normal pregnancy; the difference, however, is not significant. That trend is in agreement with that reported by Yamamoto (1967) in his studies on plasma insulin in pre-eclamptic women except that normal pregnant patients were not available for comparison in his study. Madsen *et al* (1973), however, found that fasting plasma insulin levels were the same in non-pregnant patients, in normal pregnancy and in "slight pre-eclampsia". His diagnostic criteria for pre-eclampsia, however, are at variance with most authors and would partly explain the discrepancy.

Following an intravenous glucose load, the insulin response in the severe pre-eclampsia group was less than in the mild pre-eclampsia and normal pregnancy groups. Similar observations were made by Yamamoto (1967) following oral glucose loads. It is possible that this diminished insulin response to a glucose load is responsible for the abnormal glucose tolerance noted in this study, as Lerner and Porte (1971) have shown that the disappearance rate of glucose following an intravenous glucose tolerance test is determined by the first phase insulin response.

Whereas Govan *et al* (1951) supported the theory of anterior pituitary hyperactivity, Burt (1957) suggested that corticosteroid influences might be responsible for the abnormal carbohydrate metabolism in pre-eclampsia. Although the diabetogenic effect of normal pregnancy may be related to the levels of oestrogens, progesterone, catecholamines, HPL and cortisol, the available evidence suggests that output of these hormones are within normal limits or diminished in pre-eclampsia. It appears that the most likely explanation for the apparent abnormality of β-cell function is that of anoxia subsequent to the vascular changes in pre-eclampsia.

Having demonstrated that there is a disturbance of carbohydrate metabolism in pre-eclampsia and that this disturbance is similar to that of the chemical gestational diabetic, one wonders whether or not pre-eclampsia is in some way associated with the development of diabetes mellitus in later life.

O'Sullivan *et al* (1971) in a follow-up study of 603 patients without pre-eclampsia but with abnormal glucose tolerance tests in pregnancy found that one-third had developed diabetes mellitus 15 years later. In a follow-up study carried out by Singh *et al* (1973) where intravenous glucose tolerance tests were performed on patients who had had severe pre-eclampsia 10 to 20 years previously, no significant difference was found in the incidence of abnormal increment indices compared with a control group of patients with no history of pre-eclampsia.

This study suggests that carbohydrate metabolism in severe pre-eclampsia is altered to an extent similar to that of the chemical gestational diabetic. This disturbance seems to be transient and does not appear to be associated with the later development of diabetes mellitus.

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