

Improvement of Oral Glucose Tolerance in Gestational Diabetes by Pyridoxine

H. J. T. COELINGH BENNINK, W. H. P. SCHREURS

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Summary

Fourteen pregnant women were shown by the oral glucose tolerance test to have gestational diabetes. In 13 an increased urinary xanthurenic-acid excretion after an oral load of L-tryptophan indicated a relative pyridoxine deficiency.

All patients were treated with vitamin B₆ (pyridoxine) 100 mg/day for 14 days by mouth, after which the pyridoxine deficiency disappeared and the oral glucose tolerance improved considerably. Only two patients then had sufficiently impaired glucose tolerance to justify the diagnosis of gestational diabetes.

Our results substantiated our hypothesis that increased xanthurenic-acid synthesis during pregnancy may cause gestational diabetes. Treatment with vitamin B₆ makes the production of xanthurenic-acid normal by restoring tryptophan metabolism and improves the oral glucose tolerance in patients with gestational diabetes.

Introduction

The importance of identifying and treating gestational diabetes is increasingly being recognized. Even mild gestational diabetes, which can be treated successfully by dietary measures or small doses of insulin, may without treatment become complicated by increased perinatal morbidity and mortality.¹⁻⁴ Nevertheless, the cause of impaired glucose tolerance during gestation is unknown.

It has long been known that during gestation tryptophan-nicotinic acid metabolism needs more of the rate-limiting coenzyme pyridoxal phosphate. Consequently, nearly every pregnant woman will develop a relative pyridoxine deficiency, as shown by an increased urinary excretion of tryptophan metabolites, particularly xanthurenic acid,⁵⁻⁸ after oral administration of L-tryptophan (fig. 1). The increased need for pyridoxal phosphate is met by oral administration of vitamin B₆, after which xanthurenic-acid excretion after an oral load of L-tryptophan becomes normal.⁶⁻⁷ In animal experiments xanthurenic acid has a diabetogenic effect.¹⁷⁻¹⁸ It forms a stable complex with insulin and acts as an insulin antagonist.⁸ From these data we formulated the following hypothesis: by insulin antagonistic effects xanthurenic acid disturbs maternal glucose tolerance during gestation and may cause gestational diabetes; vitamin B₆ by mouth eliminates the relative pyridoxine deficiency and thus the tryptophan-nicotinic acid metabolism is restored, xanthurenic-acid synthesis decreases, and glucose tolerance improves.

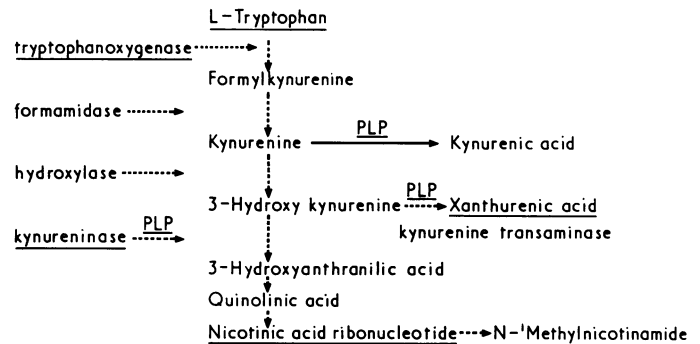


FIG. 1—Tryptophan-nicotinic acid metabolism. PLP = Pyridoxal-5-phosphate.

Patients and Methods

Fourteen healthy pregnant women had abnormal oral glucose tolerance test (O.G.T.T.) results and were diagnosed as having gestational diabetes. The O.G.T.T. had been performed for one or more of the following indications: recurrent abortion, infertility of over five years, family history of diabetes mellitus, positive urinary reducing tests, or an abnormal obstetrical history (intra-uterine death, serious toxæmia of pregnancy, congenital malformations, or birthweight of previous children of over 4000 g). All 14 patients were regularly examined at the prenatal clinic of the Utrecht University Hospital. The patients' average age was 27 years (range 22-42 years). Five women were nulliparæ, five para 1, three para 2, and one para 4.

The first abnormal O.G.T.T. result occurred after an average period of amenorrhœa of 29 weeks (range 15-33 weeks). All patients were put on a diet and pyridoxine hydrochloride (vitamin B₆) 100 mg/day given for 14 days. On the 15th day the O.G.T.T. was repeated. At that time none of the patients were being treated with insulin. The average time between the abnormal O.G.T.T. result and the O.G.T.T. after treatment with vitamin B₆ was four weeks. The average weight at the first O.G.T.T. was 65.2 kg (53.4-81 kg) and at the second 64.3 kg (53.7-78.2 kg).

Vitamin B₆ concentration in blood and the xanthurenic-acid excretion in the urine over eight hours after an oral load of 2 g L-tryptophan were determined⁹ before and after vitamin B₆ treatment.

The oral glucose tolerance test was carried out according to the standards advised by the World Health Organization.¹⁰⁻¹¹ The maximal normal glucose concentrations in the five stages of the test are shown in table I. The result was classed as abnormal when at least two values exceeded these norms. The glucose concentration was measured in capillary blood by an enzymatic method with glucose oxidase (GOD period, Boehringer). The vitamin B₆ concentration in blood was determined by the microbiological method of

TABLE I—Maximal Normal Glucose Concentrations (mmol/l) at Various Times during Oral Glucose Tolerance Test and Values Before and After Treatment with Vitamin B₆. Statistical Comparisons are between Values Before and After Treatment

	Fasting	½ h	1 h	2 h	3 h
Maximal normal concentrations	5.6	8.9	8.9	6.7	5.6
Mean values before vitamin B ₆	5.2	7.8	9.4	7.2	4.8
Mean values after vitamin B ₆	4.4	7.3	7.7	5.8	4.5
P	<0.01	N.S.	<0.01	<0.05	N.S.

Conversion: SI to Traditional Units
Glucose: 1 mmol/l ≈ 18 mg/100 ml.

Department of Obstetrics and Gynaecology, University Hospital, Utrecht, The Netherlands

H. J. T. COELINGH BENNINK, M.D., Resident

Central Institute for Nutrition and Food Research T.N.O., Zeist, The Netherlands

W. H. P. SCHREURS, PH.D., Head of Department of Biochemistry

Atkin *et al.*¹² Urinary xanthurenic-acid concentration was measured by the method of Wachstein and Gudaitis.¹³

The sign test and the Wilcoxon signed rank test were used to evaluate statistically the results of the O.G.T.T.s., $P < 0.05$ being regarded significant.

Results

Vitamin B₆ Concentration in Blood.—The average values and the range found before and after giving vitamin B₆ are shown in table II. Before the administration of vitamin B₆ the blood concentration seemed to be below normal in eight of the 14 patients, and low normal levels were found in the other six. After vitamin B₆ treatment all women showed above normal concentrations in their blood.

TABLE II—Blood Concentration of Vitamin B₆ and Xanthurenic Acid Excretion in Urine after Oral Charge of 2 g L-Tryptophan in 14 Women with Gestational Diabetes Before and After Treatment with Vitamin B₆

	Mean (Range) Blood Vitamin B ₆ ($\mu\text{g/l}$)	Mean (Range) Xanthurenic Acid Excretion in Urine ($\mu\text{mol/8 h}$)
Normal values	13.5–25.0	≤ 60
Before vitamin B ₆	12.8 (10.8–15.7)	279 (39–715)
After vitamin B ₆	83.1 (58–200)	39 (13–54)*

*54 $\mu\text{mol/8 h}$ was reached in one patient after 28 days' vitamin B₆ treatment (see case report).

The urinary xanthurenic-acid excretion over eight hours after an oral charge of 2 g L-tryptophan before and after vitamin B₆ treatment is shown in table II. Before treatment the xanthurenic-acid excretion in 13 of the 14 patients showed a slight to high increase. A normal excretion was found once (39 $\mu\text{mol/8 h}$). After treatment with vitamin B₆ this patient's xanthurenic-acid excretion did not change (41 $\mu\text{mol/8 h}$), but in the other 13 patients the excretion decreased to normal. One patient had to be given vitamin B₆ 100 mg/day during a second period of 14 days to restore the xanthurenic-acid excretion to normal.

Oral Glucose Tolerance Test.—On the initial O.G.T.T. eight patients had two glucose values above normal, the other six patients having three raised values. After the administration of vitamin B₆ the oral glucose tolerance returned to normal in six patients and showed only one value exceeding the limit in six others. In two patients there were two values on the O.G.T.T. which exceeded the limits, so as a result of vitamin B₆ treatment the diagnosis of gestational diabetes could be made in only two of the 14 patients (sign test $P < 0.01$). The average values before and after treatment are shown in table I. The total of the five glucose values decreased in 13 of the 14 patients, while it increased in one after administering vitamin B₆ (table III)—a significant difference by the Wilcoxon signed rank test ($P < 0.01$).

The influence of vitamin B₆ treatment on the vitamin B₆ concentration in blood, the excretion of xanthurenic acid in the urine, and the glucose tolerance are illustrated by the following case.

In a 27-year-old woman (para 2, gravida 3) a disturbed glucose tolerance was found after 29 weeks' amenorrhoea (fig. 2 A). The O.G.T.T. was performed because of a history of serious toxæmia of pregnancy and a family history of diabetes mellitus. The patient's weight was 72.5 kg and the vitamin B₆ concentration in the blood 10.8 $\mu\text{g/l}$. The xanthurenic-acid urine excretion after an oral load of 2 g L-tryptophan was high (715 $\mu\text{mol/8h}$). The patient was treated by diet and given vitamin B₆ 100 mg/day for 14 days. After 34 weeks' amenorrhoea the O.G.T.T. was repeated and showed one abnormal value (fig. 2 B). The patient's weight was then 70.2 kg, the blood vitamin B₆ concentration 58 ng/ml, and the urinary xanthurenic acid excretion 80 $\mu\text{mol/8 h}$. Though the last had decreased it was still too high, and hence she

was again treated with vitamin B₆ 100 mg/day for 14 days. After amenorrhoea of 36½ weeks the O.G.T.T. result was completely normal (fig. 2 C). The patient weighed 70 kg, and the xanthurenic-acid excretion in the urine was normal (41 $\mu\text{mol/8 h}$).

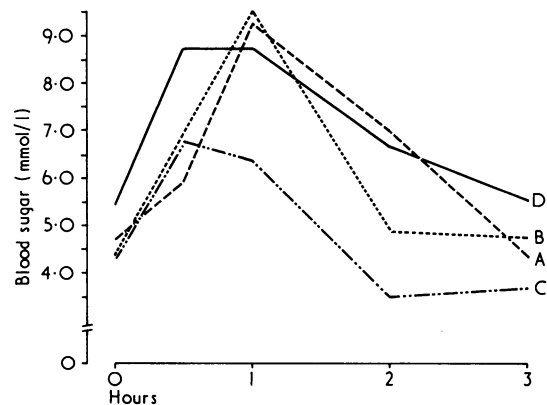


FIG. 2—Influence of vitamin B₆ on oral glucose tolerance of 27-year-old woman with gestational diabetes (A) after 29 weeks' amenorrhoea, (B) after 34 weeks' amenorrhoea, and (C) after 36½ weeks' amenorrhoea. D = Normal glucose tolerance curve.

Conversion: SI to Traditional Units—Glucose: 1 mmol/l \approx 18 mg/100 ml.

Discussion

During gestational diabetes the progressive diabetogenic influence of pregnancy is thought to be caused by insulin antagonism,¹⁴ as indicated by an increase in the serum insulin level two to three times greater than that in normal pregnant controls.¹⁵ Several substances which are present in increased amounts during gestation—namely, oestrogens, progesterone, human chorionsomatotrophic hormone, cortisone, and free fatty acids—have been mentioned as possible insulin antagonists and are thought to be connected with the pathogenesis of gestational diabetes.¹⁶ But the well-known changes which occur in tryptophan-nicotinic acid metabolism during pregnancy^{5, 6, 7} and which increase the excretion of the diabetogenic insulin antagonist xanthurenic acid^{8, 17, 18} have never been related to the pathogenesis of gestational diabetes.

In our 14 patients with gestational diabetes 13 showed an increased xanthurenic-acid excretion after an oral load of L-tryptophan, which indicated at least a relative pyridoxine deficiency, and eight patients had abnormally low blood vitamin B₆ levels. Blood vitamin B₆ levels in pregnancy are known to be reduced or deficient towards the end of gestation,¹⁹ but oral vitamin B₆ treatment over two weeks (100 mg/day) restored the vitamin B₆ status of all 13 deficient patients to normal. The glucose tolerance also improved significantly even though pregnancy had advanced about four weeks and a further deterioration of oral glucose tolerance was to have been expected.¹⁴ In our laboratory dietary measures have a negligible effect on the standardized oral glucose tolerance test. The decreases in the mean fasting and two-hour values were especially important because these values are generally thought to be the most significant ones.

In conclusion, tryptophan-nicotinic acid metabolism obviously becomes unbalanced in pregnant patients with gestational diabetes. When this balance was restored by oral administration of vitamin B₆ the increased excretion of the diabetogenic metabolite xanthurenic acid became normal in our patients,

TABLE III—Total of Five Glucose Values (mmol/l) on Oral Glucose Tolerance Test Before and After Treatment with Vitamin B₆

Case No.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Mean
Before	33.0	35.3	31.4	32.6	40.1	36.7	33.0	33.0	36.4	32.7	32.6	36.4	32.8	34.8	34.4
After	27.9	29.4	30.5	22.1	33.0	29.0	37.0	32.5	31.6	30.5	29.1	38.9	29.1	24.1	29.6
Difference	-5.1	-5.9	-0.9	-10.5	-7.1	-7.7	+4.0	-0.5	-4.8	-2.2	-3.5	-7.5	-3.7	-10.7	-4.8

while their oral glucose tolerance improved. Xanthurenic acid may act as an insulin antagonist during pregnancy and sometimes cause gestational diabetes. Adaptation of insulin production and release to increasing synthesis of an insulin antagonist during pregnancy may determine whether or not a disturbance in the glucose tolerance will result. Nevertheless, it remains to be proved whether there is a relation between xanthurenic acid and gestational diabetes. Oral administration of vitamin B₆ certainly improves significantly the oral glucose tolerance in gestational diabetes, but it is most important to know whether vitamin B₆ given prophylactically could have a long-term influence by preventing the development of gestational diabetes during pregnancy.

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Defective Innervation of Heart in Diabetic Autonomic Neuropathy

R. H. LLOYD-MOSTYN, P. J. WATKINS

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Summary

Heart rate responses to autonomic stimulation and inhibition were studied in 13 diabetics with autonomic neuropathy. Parasympathetic function was impaired in all patients and sympathetic function in most. One patient's heart appeared to be totally denervated. The consequences of cardiac denervation include tachycardia, a fixed heart rate, and a possible tendency to cardiac dysrhythmias, which caused spontaneous cardiac arrests in three patients.

Introduction

Diabetic autonomic neuropathy may have widespread consequences, and many abnormalities of the peripheral vascular system have been described.¹⁻⁴ Defective autonomic control of the heart has received much less study. Our aim was to discover whether cardiac efferent nerves are involved in diabetic neuropathy and see how far such abnormalities might account for defective cardiovascular reflexes.

Methods

Thirteen patients who showed severe diabetic complications, including features of autonomic neuropathy, were studied (see table). None had ever been in cardiac failure and only one patient's electrocardiogram showed minor ischaemic changes. The patients lay supine for at least half an hour before the investigations. Standard electrocardio-

grams were recorded, and lead II strips were taken for measurement of heart rate. In most patients instantaneous heart rate was also recorded on tape and a tracing showing beat-to-beat variation obtained with a Hewlett-Packard heart rate monitor 8020A. Continuous intra-arterial blood pressure recordings were made in nine patients. In the remaining four, blood pressure was recorded with a sphygmomanometer. Cannulation of a radial artery at the wrist was performed with a Grandjean catheter introduced by a microSeldinger technique. A direct write-out of the pressure trace was obtained on a Mingograph recorder with an Elema-Schönander pressure transducer.

The following tests were used to assess the effects of the sympathetic and parasympathetic nerves in the control of heart rate.

Mental Calculation.—Subtraction tasks were rapidly performed aloud under harassment.

Tilting.—Patients were tilted to vertical for three minutes.

Valsalva Manoeuvre.—Patients blew into a modified mercury sphygmomanometer. A pressure of 40 mm Hg was reached as soon as possible and maintained for 15 seconds. Pressure was then released abruptly and recordings were continued for at least another 20 seconds.

Phenylephrine.—Transient hypertension was induced by the intravenous injection of phenylephrine to test baroreceptor reflexes. A rise in systolic pressure of 15 mm Hg was achieved with 50 µg in all but four patients, who received an additional 100 µg.

Carotid Sinus Pressure.—Firm pressure was applied for 10 seconds to each carotid sinus in turn.

Amyl Nitrite.—Transient hypotension was induced by inhaling 1 ampoule of amyl nitrite, the systolic pressure falling by at least 20 mm Hg in all patients.

After each of these tests the pulse rate and blood pressure were allowed to return as near to control levels as possible before the next test was carried out.

Propranolol.—A 10 mg dose of propranolol was given slowly intravenously to block sympathetic stimulation of the heart.

Atropine.—Atropine 1.8 mg was given intravenously about 30 minutes after propranolol to block vagal stimulation of the heart.

Lobeline.—On the same or a different day eight patients were given 6 mg lobeline by rapid intravenous injection to test chemoreceptor reflex pathways.

Results

Basal Heart Rate.—In three patients the resting heart rate was over 95/min, and in six it was 85 or more per min (fig. 1).

Beat-to-beat variation (sinus arrhythmia) of the heart rate on deep breathing was normal in only one patient, all the others showing variations of 7 or less per min, mostly 3 or less per min. The normal beat-to-beat variation in adults under 50 years of age is always greater than 10/min.⁵

King's College Hospital, London SE5

R. H. LLOYD-MOSTYN, M.R.C.P., Research Fellow, Cardiac Department (Now Senior Registrar, Queen Elizabeth Hospital, Birmingham B15 2TH)
P. J. WATKINS, M.D., M.R.C.P., Consultant Physician, Diabetic Department