References
1. Harris MI, Robbins DC: Prevalence of adult-onset IDDM in the U.S. population. *Diabetes Care* 17:1337–1340, 1994
2. Harris MI, Hadden WC, Knowler WC, Bennett PH: Prevalence of diabetes and impaired glucose tolerance and plasma glucose levels in U.S. population aged 20–74 yr. *Diabetes* 36:523–534, 1987
3. Maislos M, Bodner-Frishman B, Weitzman S: Prevalence and clinical characteristics of type I and type II insulin-treated diabetes in the community. *Diabetes Care* 17:1230–1231, 1994

Is It Time to Modify the Glucose Tolerance Test for the Diagnosis of Gestational Diabetes?

The diagnosis of gestational diabetes mellitus (GDM) identifies two people at increased risk. The glucose levels in pregnancy that define the risk to the mother of developing non-insulin dependent diabetes at a future date are well established. However, the method of testing and the maternal glucose levels that will be most sympathetic to the fetal outcome are still to be defined.

In the November 1994 issue of *Diabetes Care*, Pettitt et al. (1) raised several important points regarding the diagnosis of GDM.

First, a 2-h glucose level ≥7.8 mmol/l after a 75-g glucose load appeared to be a better predictor of certain fetal outcomes than the National Diabetes Data Group criteria. A change to the World Health Organization (WHO) criteria during pregnancy would also facilitate comparisons with subsequent nonpregnant glucose tolerance test (GTT) results.

Second, doubts about the reliability and hence the usefulness of the fasting glucose level for the diagnosis of GDM were raised. These concerns have also been expressed by the WHO (2).

Third, nearly one-third of patients who were scheduled for a two-stage diagnostic procedure did not return for the second stage. Little importance has been given so far to the "no show" rate, which must markedly alter the incidence and the clinical reliability of testing for GDM.

These observations suggest that more consideration should be given to a testing procedure that is simple to administer from the medical side, encourages a high rate of maternal compliance, and is predictive of adverse fetal outcomes.

Since the beginning of 1993, a modification of the 75-g GTT (MGTT) has been evaluated in the Illawarra area of Australia. Women at two prenatal clinics have, over alternating 6-month periods, been offered either a GTT with both fasting and 2-h samples or an MGTT in which only the 2-h sample is taken. Both tests are administered in the morning after an overnight fast. The advantage of the MGTT is that the patient can be given the glucose solution at a preceding prenatal clinic (or doctor's office) visit and only needs to present for one blood test (3). GDM is diagnosed if the fasting plasma glucose is ≥5.5 mmol/l and/or the 2-h plasma glucose is ≥8.0 mmol/l (4).

However, even with this one-stage procedure there have been different rates of initial compliance. Ninety-four percent (707 out of 752) have completed the simpler MGTT, while only 90.1% (1,237 out of 1,367) have completed the GTT ($\chi^2 = 8.01$, df = 1, $P < 0.01$).

Apart from a 0.2 mmol/l difference in the 2-h glucose level for the diagnosis of GDM, the major difference between the MGTT and the one-stage test used by Pettitt et al. (1) is whether the woman needs to fast before the test. The argument against the need for fasting is convincing, and not fasting would further simplify the procedure.

Perhaps now is the time to revise the GTT used in pregnancy so that the glucose criteria are more sympathetic to the fetal outcome and the testing procedure is simplified to encourage the highest possible rate of maternal compliance.

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References
1. Pettitt DJ, Bennett PH, Hanson RL, Venkat Narayan KM, Knowler WC: Comparison of World Health Organization and National Diabetes Data Group procedures to detect abnormalities of glucose tolerance during pregnancy. *Diabetes Care* 17:1264–1268, 1994
2. World Health Organization: *Prevention of Diabetes Mellitus*. Geneva, World Health Org., 1994 (Tech. Rep. Ser., no. 844)
3. Moses RG: Screening for gestational diabetes mellitus (Letter). *Med J Aust* 157:500, 1992
4. Martin FIR: The diagnosis of gestational diabetes. *Med J Aust* 155:112, 1991

Insulin Sensitivity in Patients with NIDDM and the A-to-G Mutation at Nucleotide 3,243 of the Mitochondrial tRNA$^{Leu(UUR)}$ Gene

Recent studies have shown linkage between diabetes and mutations in mitochondrial DNA, the most common of which is a G-to-A transition in the tRNA$^{Leu(UUR)}$ gene at nucleotide 3,243

Table 1—*Clinical features of subjects with NIDDM and A-to-G mutation at nucleotide 3,243*

	Subject				
	1	2	3	4	5
Present age (years)	61	34	45	42	38
Sex (M/F)	F	F	M	M	M
Duration of diabetes (years)	7	5	20	8	10
Body mass index (kg/m^2)	20.5	14.2	18.0	14.8	16.0
GIR (mg · kg^{-1} · min^{-1})	5.97	2.90, 2.99	5.88	7.50	6.57
HbA_{1c} (%)	6.6	9.9, 8.3	8.2	7.3	7.2
Treatment	OHA	OHA	INS	INS	INS
Hearing loss	−	+	+	+	+
Muscle weakness	−	+*	−	−	+
Arrhythmia	−	+	−	−	+
Stroke-like episodes	−	−	−	−	+
Proteinuria	−	−	+	+	+
Lactate (mmol/l)	1.12	2.74	1.26	3.2	0.97
Pyruvate (μmol/l)	103	119	55	148	60
Lactate:pyruvate ratio	10.8	23.0	22.9	21.6	16.1

Subject 1 is the mother of subject 2; and subjects 3 and 4 are brothers. Normal ranges: lactate, 0.44–1.78 nmol/l; pyruvate, 34–102 μmol/l; lactate:pyruvate ratio, 10. OHA, oral hypolglycemic agent; INS, insulin. The presence of the A-to-G mutation at nucleotide 3,243 was determined by polymerase chain reaction (PCR) and digestion with *Apa*I (7). Genomic DNA was prepared from peripheral blood lymphocytes. An 853-bp fragment encompassing the mitochondrial $tRNA^{Leu(UUR)}$ gene was amplified using PCR in the presence of [α-^{32}P]-dCTP. The 853-bp ^{32}P-labeled PCR product was digested with *Apa*I, which cleaves the mutant sequence into fragments of 640 and 213 bp, which were separated by electrophoresis on a 5% nondenaturing polyacrylamide gel. * Subject 2 also had histological evidence of mitochondrial myopathy with ragged red fibers.

(1–3). Clinically, subjects with diabetes due to a mitochondrial mutation present a variable clinical picture, ranging from mild glucose intolerance to a slowly progressive form of insulin-dependent diabetes mellitus (IDDM). These subjects also commonly have hearing loss and, in rare instances, symptoms of mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) (2,3). Previous studies have pointed to β-cell dysfunction as the common feature in subjects with diabetes and mitochondrial mutations, and they have suggested that this is the primary cause of diabetes. The effect of mitochondrial mutations, which are expressed in all tissues, although to varying degrees, on insulin sensitivity in peripheral tissues has been less clearly defined. Euglycemic clamp studies in two subjects, one with IDDM and one with non-insulin-dependent diabetes mellitus (NIDDM), suggested that glucose uptake in peripheral tissues was not impaired (4). Similar studies in a subject with NIDDM and MELAS showed evidence of insulin resistance (5). We therefore used the euglycemic-hyperinsulinemic clamp technique to study insulin sensitivity in five subjects from three families with an A-to-G mutation at nucleotide 3,243 of the mitochondrial $tRNA^{Leu(UUR)}$ gene.

Twenty-four subjects from 17 families with NIDDM and hearing loss who were attending the Diabetes Center of Tokyo Women's Medical College were tested for the presence of the A-to-G mutation at nucleotide 3,243. This mutation was present in 12 subjects from six families. Five subjects from three families consented to studies of insulin sensitivity using the euglycemic-hyperinsulinemic clamp method. The clinical features of these five subjects are presented in Table 1. In addition to NIDDM and hearing loss, subjects also had mitochondrial myopathy and cardiac arrhythmia (subject 2) and MELAS (subject 5). All showed an inadequate insulin or C-peptide response to the prevailing level of hyperglycemia during a 75-g oral glucose tolerance test or meal test (data not shown). The euglycemic-hyperinsulinemic clamp was performed using an artificial endocrine pancreas (Nikkiso STG-22, Nikkiso, Tokyo) after overnight fasting. A primed continuous intravenous insulin infusion was administered at a rate of 1.2 μU · kg^{-1} · min^{-1}, and the venous plasma glucose level was maintained at 4.4 mmol/l by infusing 10% glucose according to the algorithm of DeFronzo et al. (6). Blood samples were also taken for measurement of plasma levels of pyruvate and lactate at the time of the clamp study. During steady-state hyperinsulinemia (plasma insulin level, 454 pmol/l), the glucose infusion rate (GIR) varied from 2.90 to 7.50 mg · kg^{-1} · min^{-1} (Table 1). The values for normal healthy Japanese subjects

(n = 21) and subjects with NIDDM (n = 135) were 6.81 ± 1.65 and 3.79 ± 2.20 mg · kg^{-1} · min^{-1} (mean ± SD), respectively (T.W. et al., unpublished observations). The GIR values obtained for subjects 1, 3, 4, and 5 were in the normal range, indicating normal insulin sensitivity. However, the GIR value in subject 2 was in the range found in subjects with the common late-onset form of NIDDM, indicating moderate insulin resistance. Subject 2 was tested on two occasions separated by 8 months with similar results, 2.90 and 2.99 mg · kg^{-1} · min^{-1}, respectively, although at the time of retesting she showed improvement in muscle strength and HbA_{1c} levels (Table 1).

These data, which demonstrate insulin resistance in only one of five subjects with the A-to-G mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene, are consistent with the conclusion that insulin resistance is a minor component in the pathophysiology of the diabetic syndrome in these subjects. The cause of the insulin resistance is not known. It does not appear to be secondary to the presence of hyperglycemia since the degree of insulin resistance in subject 2 was constant despite a fall in the HbA_{1c} levels from 9.9 to 8.3%. Furthermore, subject 3 showed normal insulin sensitivity with HbA_{1c} value of 8.2%, which is very similar to that seen in subject 2 at the time of the second study. It is also unlikely to be related to mitochondrial myopathy, since subject 5 had MELAS and normal insulin sensitivity, or serum lactate/pyruvate levels, since subject 4 had higher levels of both these metabolites and no impairment of insulin-stimulated glucose uptake. The moderate insulin resistance noted in subject 2 could reflect normal population variation with this subject having decreased insulin sensitivity for reasons other than the presence of a mitochondrial mutation. Prospective studies of these and other subjects with mitochondrial mutations are necessary to address this important issue. In contrast, all five subjects tested demonstrated inappropriately low insulin secretory responses to glucose in the face of hyperglycemia consistent with the primacy of the β-cell lesion in the pathophysiology of the hyperglycemia present in this condition.

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References

1. van den Ouweland JMW, Lemkes HHPJ, Ruitenbeek W, Sandikuijl LA, de Vijlder MF, Struyvenbreg PAA, van de Kamp JJP, Maassen JA: Mutation in mitochondrial $tRNA^{Leu(UUR)}$ gene in a large pedigree with maternally transmitted type 2 diabetes mellitus and deafness. *Nature Genet* 1:368–371, 1992
2. Oka Y, Katagiri H, Yazaki Y, Murase T, Kobayashi K: Mitochondrial gene mutation in islet-cell-antibody-positive patients who were initially non-insulin-dependent diabetics. *Lancet* 342:527–528, 1993
3. Remes AM, Majamaa K, Herva R, Hassinen IE: Adult-onset diabetes mellitus and neurosensory hearing loss in maternal relatives of MELAS patients in a family with the $tRNA^{Leu(UUR)}$ mutation. *Neurology* 43:1015–1020, 1993
4. Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto Y, Hayakawa T, Matsuoka K, Kawamori R, Kamada T, Horai S, Nonaka I, Hagura R, Akanuma Y, Yazaki Y: A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N Engl J Med* 330:963–968, 1994
5. Kanamori A, Tanaka K, Umezawa S, Matoba K, Fujita Y, Iizuka T, Yajima Y: Insulin resistance in mitochondrial gene mutation. *Diabetes Care* 17:778–779, 1994
6. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
7. Iwasaki N, Ohgawara H, Nagahara H, Kawamura M, Bell GI, Omori Y: Characterization of Japanese families with early-onset type 2 (non-insulin dependent) diabetes mellitus and screening for mutations in the glucokinase and mitochondrial $tRNA^{Leu(UUR)}$ genes. *Acta Diabetologica* 32: 17–22, 1995

Five Cases of Hyperthyroidism in Type I Diabetic Patients Treated With Intraperitoneal Insulin Infusion

Intraperitoneal insulin infusion (IPII) using programmable implantable devices has been shown to be feasible and safe since it decreases patients' HbA_{1c} levels while reducing their risk of severe hypoglycemia (1).

From 1989 to early 1994, 62 C-peptide–negative type I diabetic patients were implanted with a pump in our center; the pumps use the Genapol-stabilized Hoescht 21PH insulin (Hoechst. AG). Ultrasensitive thyrotropin (TSH) serum concentrations (0.15–4.5 μU/ml) were performed every 6 months using an immunochemiluminometric assay (Beri-Lux). Anti-insulin antibodies (AIAs) were assessed every 3 months (n < 1.5%) by radioimmunoassay. Antimicrosomal antibodies (n < 100 U/ml), TSH-receptor antibodies (n < 10 U/ml) and free T_4 levels (n = 9–20 pg/ml) were assessed when needed.