femoral neck was done at 2 weeks (baseline 1), 3 and 6 months post-partum, and 2 weeks after the subsequent birth (baseline 2). Additional measurements were made at 12 months post-partum and 3 months post-lactation unless the woman had already conceived. At enrolment, the women averaged 33 (SD 3) years, parity 1.2 (SD 0.4), height 1.66 (SD 0.06) m, weight 69.6 (SD 11.5) kg, and had a calcium intake of 39.9 (SD 14.3) mmol daily (1590 [SD 570] g daily). All the women breastfed for at least 3 months* five for lactating; three within 2 months of the 6-month bone measurement.

Lactation was associated with a significant decrease in bone mineral status (table). However, there was no significant difference between baseline 1 and baseline 2, indicating that the bone loss had recovered before or during the subsequent pregnancy (table). For the nine mothers who conceived after 12-months post-partum, recovery had taken place in later lactation or in the post-lactation period: there were no significant differences in bone mineral status (table). However, there was no significant difference between baseline 1 and baseline 2.

Lactation was associated with a significant decrease in bone mineral status (table). However, there was no significant difference between baseline 1 and baseline 2, indicating that the bone loss had recovered before or during the subsequent pregnancy (table). For the nine mothers who conceived after 12-months post-partum, recovery had taken place in later lactation or in the post-lactation period: there were no significant differences in bone mineral status (table). However, for the three women who conceived soon after the 6-month measurement while still lactating, restoration of bone mineral status occurred during pregnancy itself (change between 6 months and baseline 2: spine =+2.0%, SE 0.06%, p=0.02; femoral neck =+4.3%, SE 0.12%, p=0.02; whole body =+1.3%, SE 0.6%, p=0.26). One mother continued to lactate throughout her subsequent pregnancy without any apparent detriment to her bone mineral status.

Our study shows that, although significant decreases in bone mineral occur during breastfeeding, these changes are reversible and do not persist after a subsequent pregnancy. This appears to be the case even when conception occurs during lactation at a time when bone loss is still evident. Thus, extended periods of breastfeeding and closely spaced pregnancies are unlikely to have a lasting effect on the bone mineral status and osteoporosis risk of healthy well-nourished women.

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Marker for liver damage in neonates born to mothers with HELLP syndrome

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The syndrome of haemolysis, elevated liver enzymes and low platelets (HELLP) is a major cause of maternal and perinatal morbidity and mortality. The aetiology and the characteristic pattern of maternal hepatic damage in this condition are not known. Deterioration of the mothers' condition is a major determinant of the management of these pregnancies, and often results in assisted delivery of a premature infant. Little is known about the consequences of this disorder in the neonate. Thrombocytopenia has not been shown in neonates born from hypertensive pregnancies with associated maternal thrombocytopenia.

Plasma glutathione S-transferase alpha 1-1 (GSTA1-1) is a sensitive indicator of hepatocellular damage in various hepatic disorders and the HELLP syndrome. To assess possible fetal hepatic damage we measured GSTA1-1 concentrations in maternal blood plasma and corresponding neonatal arterial umbilical plasma samples in nine pregnancies complicated by the HELLP syndrome and in 11 uncomplicated normotensive pregnancies. All pregnancies resulted in caesarean section.

Plasma GSTA1-1 concentrations were measured by an enzyme-linked immunosorbent assay, with an anti-human GSTA mouse monoclonal as catching antibody, and rabbit anti-human GSTA antiserum as the source of detecting antibodies. The assay has no cross-reactivity with other class GSTs and the variations within and between assays were 2.5% and 7.3%, respectively. Groups of individuals were compared with the Wilcoxon-Mann-Whitney-U test.

Maternal plasma GSTA1-1 concentrations were much higher in patients than in controls (median 37.7 and 1.3 µg/L, respectively, p=0.0001; figure). By contrast, babies born to mothers with HELLP showed similar plasma GSTA1-1 concentrations to those born to controls (4.2 and 5.9 µg/L, respectively). These results suggest that in HELLP syndrome, hepatocellular damage occurs only in the mother, and there is no evidence for neonatal hepatic damage. Toxic substances, oxygen radicals, or humoral factors may cause the maternal disease and endothelial damage. Since we
found no evidence of hepatocellular damage in neonates born from pregnancies complicated by the HELLP syndrome, these substances either do not seem to cross the fetoplacental barrier, or the fetus may not be susceptible to their effects.


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Vascular endothelial growth factor in diabetic retinopathy

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New-vessel formation in diabetic retinopathy is regulated by various angiogenic molecules. Vascular endothelial growth factor (VEGF) is involved in intraocular neovascularisation caused by retinal ischaemia, including diabetic retinopathy.1 The concentration of VEGF in intraocular fluid in patients with diabetes has been reported to be increased in proliferative diabetic retinopathy. There is a correlation between expression of VEGF and blood-retinal barrier breakdown in the streptozotocin-induced experimental diabetic rat retina.2

VEGF concentration in anterior chamber aqueous humor of the eyes in 55 patients with diabetes and five without diabetes was measured with ELISA (Quantikine Human VEGF Immunoassay kit, R&D system, MN, USA). 37 patients had non-proliferative diabetic retinopathy, nine had active proliferative retinopathy, and nine had quiescent proliferative retinopathy. Aqueous humor was obtained during cataract surgery after securing permission from patients. VEGF concentrations were compared with grade of diabetic retinopathy, retinal vessel barrier function evaluated by fluorescein angiography, duration of diabetes, blood glucose control (HbA1c), and protein concentration in aqueous humor. Aqueous protein concentration is an indicator of blood-ocular barrier function and is measured by fluorescein angiography, duration of diabetes, blood glucose control (HbA1c), and protein concentration in aqueous humor. Aqueous protein concentration is an indicator of blood-ocular barrier function and is measured with a laser-flare cell meter. 2

Multivariate regression analysis showed that VEGF concentration was significantly correlated with the grades of retinopathy and the protein concentration in aqueous humor. Mean concentration of VEGF in active proliferative diabetic retinopathy patients was 0.47 (SD 0.49) ng/mL, significantly higher than in non-diabetic patients; in patients with non-proliferative retinopathy, or with quiescent proliferative retinopathy: 0.09 (0.06) ng/mL, 0.11 (0.09) ng/mL, and 0.24 (0.10) ng/mL. In patients with non-proliferative retinopathy, VEGF concentrations tended to be higher when duration of diabetes was longer (correlation coefficient=0.31). VEGF concentration was higher in the eyes of patients with non-proliferative retinopathy compared with undamaged eyes (0.20 [0.13] ng/mL, six eyes vs 0.12 [0.05] ng/mL, seven eyes, p<0.01).

These results suggest that agents blocking VEGF may be useful to prevent progression of retinal vessel damage in diabetic retinopathy.


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BCG vaccine in insulin-dependent diabetes mellitus

Paolo Pozzilli on behalf of the IMDIAB Group*

In 1994, Shehadeh and colleagues1 reported in this journal that a single injection of Bacille Calmette Guerin (BCG) vaccine induced clinical remission in 65% of recent-onset insulin-dependent diabetes mellitus (IDDM) patients compared with 7% of controls. The number of patients included in that pilot trial was small (17). A commentary in the same issue concluded that a large trial should be undertaken without delay.2 Following previous trials aimed at increasing the remission rate in patients with recent-onset IDDM with several different approaches,3,‘ the IMDIAB V trial was set up to test the effect of BCG.

This was a randomised multicentre trial in 72 newly diagnosed IDDM patients (duration of disease less than 4 weeks, mean age 14.5 years, SD 6) aimed at comparing the effect of BCG plus nicotinamide (NCT) (25 mg per kg bodyweight) to that of the same dose of NCT alone. The number of patients to be included in the study was calculated assuming a 65% remission rate in the BCG-treated group1 and a 26% remission rate in the NCT group (the latter calculated from data presented in refs 3 and 4), setting alpha equal to 0.05 and beta equal to 90%. The vaccine was administered at IDDM diagnosis in the forearm in one single intracutaneous dose of 0.1 mL freeze-dried BCG vaccine (Berna Institute, Basel) after reconstitution with buffered saline to a final concentration of 1 mg/mL. All patients underwent intensive insulin therapy with three to four insulin injections a day as described in our previous studies.4

Results of metabolic control (insulin dose, C-peptide, glycosylated haemoglobin) at 3, 6, 9, and 12 months after diagnosis are shown in the table. No significant differences were seen between the two groups of patients. With the criteria for clinical remission reported by Shehadeh et al1 (<0.2 IU of insulin with fasting and post-prandial blood glucose below 8.3 and 11.1 mmol/L, respectively), the BCG+NCT group did not differ from the group treated with NCT in the rate of clinical remission (41% vs 46%, respectively). The length of remission was also similar between the two groups. Furthermore, these values of remission are within the expected rates even in patients receiving intensive insulin treatment without any other adjuvant therapy.

In conclusion, our data do not support the finding that BCG vaccination induces long-term clinical remission in patients with recent-onset IDDM. However, it should be underlined that, in our study, the efficacy of the BCG vaccine

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