plasma glucose and insulin concentrations were 10.3 mmol/l and 1.33 pmol/l. She was diagnosed with diabetes and insulin therapy was started. Her younger sister was also found to have glucosuria at 11 years of age during the course of a school health examination. The mother, currently 42 years of age, has been treated for diabetes since she was 27 years of age. None of the mother's three siblings is known to have diabetes but her father had been treated for diabetes. The presence of renal cysts and left renal dysplasia in the mother in addition to diabetes prompted us to screen the *HNF-1β* gene for mutations. DNA was prepared from the peripheral blood after signed informed consent was obtained from these three patients. The minimal promoter and 9 exons of the *HNF-1β* gene were screened for mutations by direct sequencing of the polymerase chain reaction products as previously described (1). We identified a G-to-A mutation of the splice donor site of exon 2 (designated IVS2nt + 1G > A) that was present in the heterozygous state in the three subjects. This mutation, which is located at the boundary between exon 2 and intron 2, changes the conserved GT-dinucleotide at the splice donor site to AT and would affect synthesis and processing of *HNF-1β* mRNA. This substitution was not found in 100 normal chromosomes.

As noted in Table 1, the mother of the subject had right renal cysts and dysplasia of the left kidney. Bilateral renal cysts 10 mm in diameter were observed in the subject and her younger sister. In addition, we observed increased cortical echogenicity and poor differentiation of corticomedullary junction in both sisters indicating some renal developmental defect. The results of various renal function tests, including renal tubular function in the mutation carriers, are summarized in Table 1: some were in the normal range whereas others were higher. The presence of renal dysfunction with no evidence of diabetic retinopathy or neuropathy suggests that the observed abnormalities are not a consequence of hyperglycaemia itself.

The subject's mother also had a bicornuate uterus. The subject and her sister were not examined for internal genital malformations. This is the second family in which a female carrier of a mutation in *HNF-1β* gene has been reported to have an abnormality related to the development of Müllerian system which gives rise to the uterus and upper vagina. The combination of anomalies of the Müllerian duct with developmental errors of the urinary tract are features of the condition termed hereditary urogenital dysplasia (OMIM 191 830). The variable expression of mutations in the *HNF-1β* gene could result in a diverse clinical phenotype.

Sincerely yours,
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C Peptide and insulin do not influence plasma semicarbazide-sensitive amine oxidase activity

Dear Sir,
In diabetes mellitus plasma activities of semicarbazide-sensitive amine oxidase (SSAO) have been reported to be raised in childhood Type I (insulin-dependent) diabetes mellitus at first clinical diagnosis [1]. SSAO belongs to a group of enzymes which convert primary amines into aldehydes, hydrogen peroxide and ammonia; increased plasma SSAO concentrations have been shown to have deleterious effects on vascular endothelium [2]. The precise physiological role of SSAO is not fully understood but it plays a part in glucose transportation into adipocytes through the GLUT4 transporter [3]. The origin of plasma SSAO and the cause of the rise in activity in pathological conditions like diabetes mellitus and congestive heart failure are not known. It has been suggested that naturally occurring inhibitors or activators or both are responsible for such changes [4, 5]. In newly onset Type I diabetes a drastic fall in both C peptide and insulin occurs, while Type II (non-insulin-dependent) diabetes mellitus usually has increased insulin and C-peptide concentrations. We wondered whether C peptide and insulin might have inhibitory or activating effects on SSAO, and that the rise in SSAO could be explained by the changing concentrations of these compounds.

SSAO activity was determined in plasma by a functional assay measuring the amount of benzaldehyde generated by SSAO from the substrate benzylamine during a 1-h incubation period [6]. Activities were measured in plasma as such, and in the same plasma to which C peptide (Eli Lilly) or insulin (Actrapid) or both had been added, each in 6 different concentrations ranging from 0.5 nmol/l to 50 μmol/l, so that $3 \times 7 = 21$ SSAO activities were determined for each plasma sample. Experiments were done with 6 different plasma samples: 2 control pools (with normal and high SSAO activity, 388 and 720 mU/l respectively), 2 from patients with diabetes mellitus (336 and 1132 mU/l) and 2 from patients with chronic heart failure (332 and 1089 mU/l). No effects of C peptide, insulin or a combination of both were seen. The 21 measurements in each plasma sample averaged 394 ± 28, 717 ± 41, 339 ± 25, 1099 ± 67, 339 ± 14 and 1074 ± 49 mU/l, respectively. The average variation coefficient was 5.9 % (range 4.3–7.5 %), not dif-

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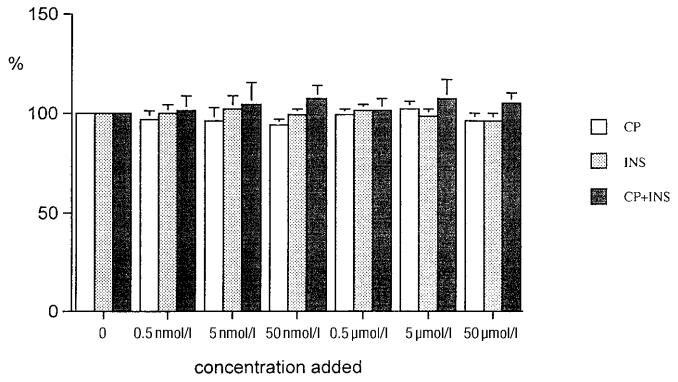

Fig. 1. Plasma SSAO activities in the absence and in the presence of various concentrations of C peptide or insulin or both. Data (mean ± SD) are expressed as a percentage of the (mean) value in the absence of added compounds

ferent from the previously established intra-assay variation coefficient.

Results of the measurements, expressed as a percentage of the mean value measured in the absence of C peptide and insulin, are given in Figure 1, and clearly show that measured SSAO activity does not change in the presence of added C peptide, insulin or both.

We conclude that neither C peptide nor insulin is an inhibitor or activator of plasma SSAO and thus that changes in concentrations of these compounds cannot explain the observed rise in plasma SSAO in diabetes. Of course, the present in vitro findings do not rule out indirect in vivo effects of insulin or C peptide on plasma SSAO activity.

Yours sincerely,

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Mitochondria from human trophoblast and embryonic liver cells are resistant to hyperglycaemia-associated high-amplitude swelling

Dear Sir,

Accumulating evidence points towards free oxygen radical overproduction participating in the induction of embryonic dysmorphogenesis in diabetic pregnancy. The excess free oxygen radical production is a consequence of glucose stimulation of the mitochondrial electron transport chain [1]. In diabetic rat pregnancy, mitochondrial morphology is altered in ectodermal cells of day-9 embryos and in the neuroepithelium of day-10 embryos, but not in the brain, heart and liver of day-15 fetuses. Similarly, embryos cultured in 50 mmol/l D-glucose but not L-glucose, exhibited high-amplitude mitochondrial swelling. Treatment with antioxidants prevented the swelling both in vivo and in vitro, suggesting that free oxygen radicals are involved in the hyperglycaemia-induced mitochondrial effects [2, 3].

To clarify whether changes in mitochondrial morphology induced by diabetes-associated hyperglycaemia are a general process in embryonic tissues, we have investigated mitochondrial morphology at the ultrastructural level in tissues of human fetal and placental origin. Primary cultures of placental trophoblast from first trimester (weeks 8–12) and term pregnancies (weeks 38–41) [4], choriocarcinoma cell lines (JAR, JEG-3 and BeWo) and a fetal hepatocyte cell line (WRL-68) were cultured for 24 h and 48 h in DMEM with 5.5 mmol/l D-glucose (normoglycaemia), 25 mmol/l D-glucose (hyperglycaemia) or 19.5 mmol/l D-mannitol + 5.5 mmol/l D-glucose (osmotic control). Cells were fixed in 2.5% glutaraldehyde in 0.1 mol/l sodium cacodylate buffer pH 7.3 for 2 h, then scraped off the base of the dish and embedded in serum [5] after which they were processed into epoxy resin. Placental tissue explants from third trimester placentas [6] were similarly incubated from 4 to 7 days in 25 mmol/l D-glucose to study the possible paracrine influences of extracellular matrix and tissue integrity. The explants were not functionally affected by hyperglycaemia with respect to hCG and LDH release or rubidium efflux (data not shown). After culture the explants were fixed as

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