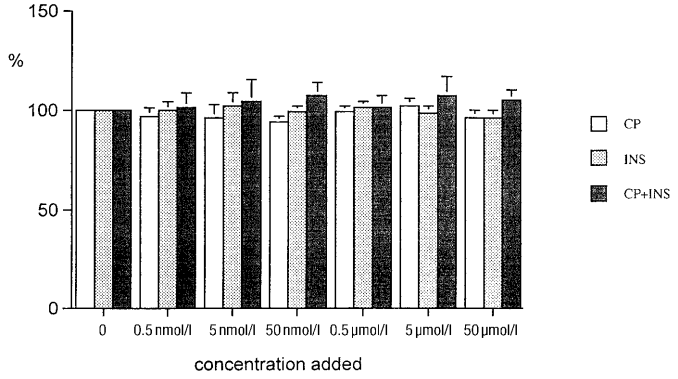

Fig. 1. Plasma SSAO activities in the absence and in the presence of various concentrations of C peptide or insulin or both. Data (mean ± SD) are expressed as a percentage of the (mean) value in the absence of added compounds

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,

F. Boomsma, K. Ekberg, G. J. Bruining

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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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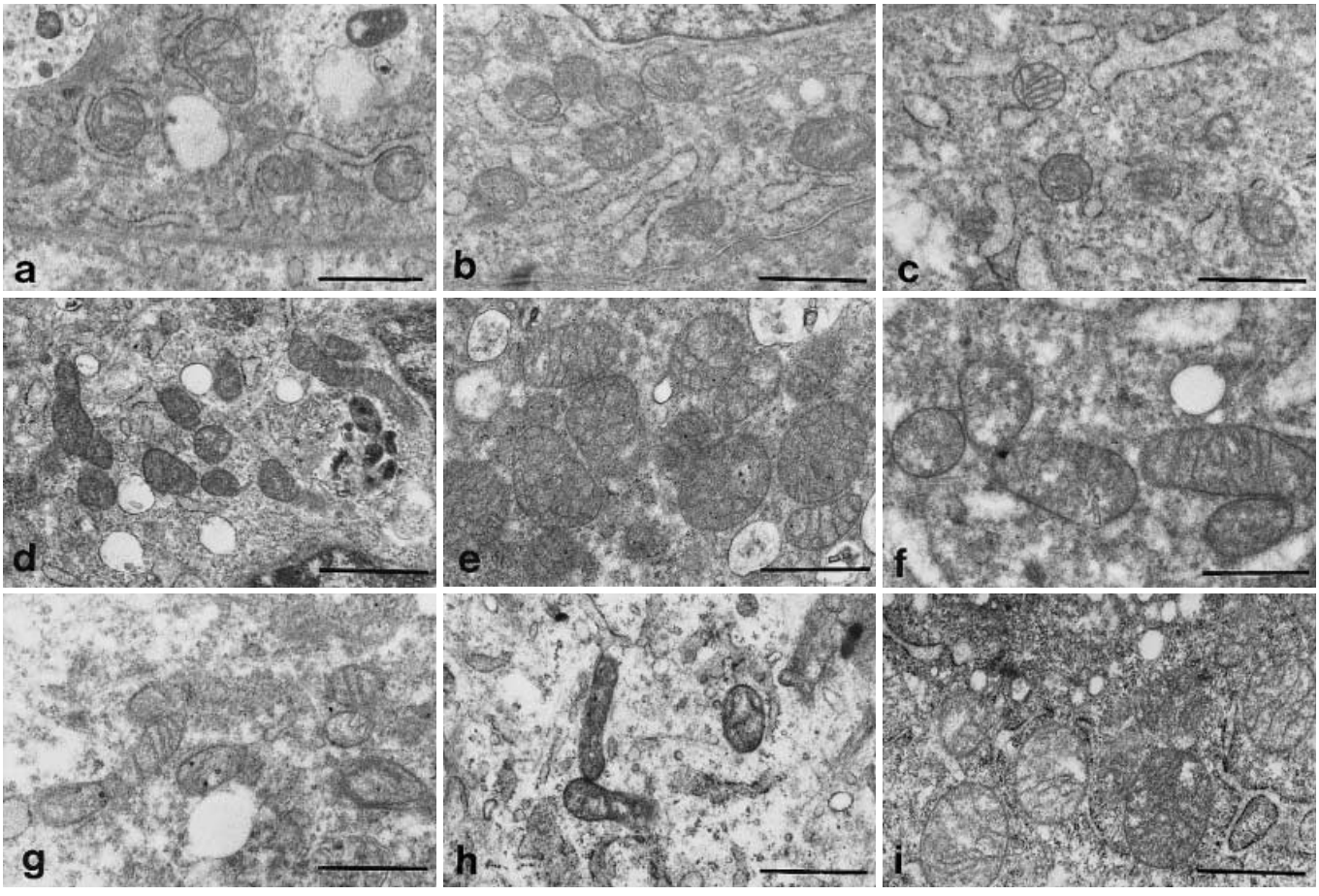

Fig. 1. Electron micrographs at identical magnification showing mitochondria of trophoblast cells from 8 week placenta exposed to a) 5.5 mmol/l D-glucose (normoglycaemia), b) 25 mmol/l D-glucose (hyperglycaemia), and c) 19.5 mmol/l D-mannitol + 5.5 mmol/l D-glucose (osmotic control) for 48 h; d) term placental trophoblast, e) JAR cells, f) JEG-3 cells, g) BeWo cells, h) placental explant tissue, i) WRL-68 hepatic cell line. d)–i) exposed to 25 mmol/l glucose (hyperglycaemia) for 48 h except for the explant culture which was incubated for 7 days. There is no evidence of high amplitude mitochondrial swelling in any of the preparations. Scale bar represents 1 μm

above, diced into 2 or 3 pieces and embedded in epoxy resin. Ultrathin sections were cut, contrasted with uranyl acetate/lead citrate and examined in a Philips 301 electron microscope (Eindhoven, Holland).

In all cases, examination of the entire ultrathin section showed that mitochondria did not appear to be influenced by hyperglycaemic conditions, in the cell cultures from the first trimester and term placentas (Fig. 1). Even malignant transformation of the trophoblast did not increase the susceptibility of the mitochondria to the hyperglycaemic insult, because in trophoblast-derived JAR, JEG-3 and BeWo choriocarcinoma cells no morphological changes of the mitochondria were identified (Fig. 1). There were inherent differences between the three cell lines, with JAR cells containing mitochondria that were generally larger and rounder than those of JEG-3, while BeWo cell mitochondria were smaller than those of both the other two cell lines, and contained fewer cristae. Absence of morphological alterations are not an in vitro artefact resulting from tissue disintegration or loss of cell-cell and cell-extracellular matrix contact, because mitochondria in the explant model were also resistant (Fig. 1). Also, after culture in hyperglycaemic conditions, there was no evidence of nuclear changes indicative of apoptosis in any of the cell lines nor any apparent increase in its occurrence in placental trophoblasts or intact tissue. To test whether the absence of effect is restricted to cells of the placenta proper or is a more general feature of the human fetus, the hepatocyte cell line (WRL-68) derived from an embryonic liver was included. Like in rat liver cells [3] no change in mitochondrial morphology could be detected, even though after 48 h many cells in all three conditions of culture showed vacuolation and swelling of cytoplasmic endomembranes (Fig. 1).

Superoxide free radical production has been observed in human trophoblast [7] and human fetal liver cells (Weiss, Pürstner and Desoye; unpublished results) cultured in high glucose concentrations. Therefore, mitochondrial high-amplitude swelling is not a general result of oxidative stress in human embryonic tissues. The absence of swelling found here can be explained by differences in glycaema, species and/or developmental stage, as compared with other findings [2, 3]. Current data, however, are not conclusive in distinguishing between these possibilities. The glucose concentration chosen here is the standard concentration used in vitro to mimic long-standing hyperglycaemia of poorly-controlled or uncontrolled diabetic mothers. It cannot be ruled out, however, that a pharmacological glucose dose might have an effect on the mitochondria. The impaired activity of mitochondrial dehydrogenases, i.e. of mitochondrial function, under hyperglycaemia in the choriocarcinoma cell models [8] suggests a stage-de-

pendent and tissue-dependent differential sensitivity of mitochondrial swelling rather than of mitochondrial function in general. Thus different mitochondrial properties appear to have differential sensitivity towards hyperglycaemia. Without knowing the molecular mechanism(s) linking free radicals to mitochondrial swelling this cannot be addressed experimentally.

We conclude that hyperglycaemia-induced high-amplitude swelling is not a general phenomenon in embryonic tissues.

Yours sincerely,

C.J.P. Jones, U. Weiss, C.M. Simán, G. Desoye

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Erratum

High glucose-induced intercellular adhesion molecule-1 (ICAM-1) expression through an osmotic effect in rat mesangial cells is PKC-NF-$\varkappa$B-dependent

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