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Supplementary webappendix

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Supplement to: McCance DR, Holmes VA, Maresh MJA, et al. Vitamins C and E for prevention of pre-eclampsia in women with type 1 diabetes (DAPIT): a randomised placebo-controlled trial. *Lancet* 2010; published online June 26. DOI:10.1016/S0140-6736(10)60630-7.

Methodology for Biological Sample Analyses in the DAPIT Trial

Biological samples, collected at baseline, 26 weeks' and 34 weeks' gestation, were stored at -70°C and batch analyzed at the end of the study in the central laboratory, Queen's University Belfast. Plasminogen activator inhibitor type 1 (PAI-1) and type 2 (PAI-2) were determined by enzyme-linked immunosorbent assay (ELISA), with samples measured in duplicate (Biopool TintElize, Trinity Biotech plc., Bray, Ireland and American Diagnostica Inc., Stamford, CT, respectively). Plasma ascorbate levels were measured using a fluorometric assay¹ in plasma stabilized with metaphosphoric acid on a Cobas FARA centrifugal analyzer (Roche Diagnostics, Switzerland). Serum concentrations of α -tocopherol were measured by high-performance liquid chromatography (HPLC) with UV detection at 292nm,² and expressed per mmol of serum cholesterol.³ Assays were standardized against appropriate National Institute of Standards and Technology reference materials. Serum total cholesterol was measured by standard enzymatic assays (Randox, Crumlin, Northern Ireland) on an automated ILab-600 biochemical analyzer (Instrumentation Laboratories, Warrington, United Kingdom). Microalbumin and creatinine (Randox, Crumlin, Northern Ireland) were measured by spectrophotometry on the automated ILab600 biochemical analyzer. HbA1c (Diazyme Labs., Poway, CA, USA) was measured by spectrophotometry on the automated ILab600 biochemical analyzer.

References:

1. Vuillemier JP, Keck E. Fluorometric assay of vitamin C in biological materials using a centrifugal analyser with fluorescence attachment. *J Micronutrient Anal* 1989; **5**: 25-34.
2. Craft NE. Carotenoid reversed-phase high-performance liquid chromatography methods: reference compendium. *Methods Enzymol* 1992; **213**: 185-205.
3. Gey KF. Ten-year retrospective on the antioxidant hypothesis of arteriosclerosis: Threshold plasma levels of antioxidant micronutrients related to minimum cardiovascular risk. *J Nutr Biochem* 1995; **6**: 206-36.