

ApoE-protein levels were determined in 2013 from stored plasma obtained at the first Health ABC follow-up visit (median time from baseline of 53.4 weeks; 25<sup>th</sup>–75<sup>th</sup> percentile 51.0–58.1 weeks). Plasma was stored at –70° C at Fisher BioServices, Inc. Laboratories and shipped directly to the analytical laboratory. ApoE-protein levels were measured by the laboratory of Dr. Rob Veerhuis at the VU University Medical Center, Amsterdam, the Netherlands, using an Elisa from Mabtech (MABTECH AB Nacka Strand, Sweden). An affinity-purified polyclonal sheep antihuman apoE antibody (ShoE/E, obtained by genetic immunization of sheep followed by boosting of animals with human apoE) was coated overnight onto MaxiSorp immune plates (Nunc Intermed, Roskilde, Denmark) (dilution 1:10<sup>3</sup> in PBS [pH 7.4]; 100 µl/well) at 4 °C. Plates were washed three times with PBS containing 0.05% Tween-20 (v/v) (PBS-T), and unspecific binding sites were blocked for 1 h at 37 °C with blocking buffer (PBS containing 0.1% casein). Plates were washed three times with PBS-T, and 100 µL of the samples, reference sera (both dilution 1:8,000), and standards (plasma from C57Bl/6 mice spiked with 0–32 µg/l of human apoE) were added (all diluted in blocking buffer). The plates were incubated overnight at 37 °C. Plates were washed five times with PBS-T to remove unbound and/or nonspecifically bound proteins, and captured antigen was detected by adding 100 µL of horseradish peroxidase (HRP)-conjugated polyclonal sheep antihuman apoE antibody (dilution 1:10<sup>3</sup> in blocking-buffer containing 0.05% Tween-20). After a 2 h incubation at 37 °C, plates were washed five times with PBS-T and 100 µl/well of freshly prepared tetramethylbenzidine (Organon Teknika, Boxtel, The Netherlands) was added, and the plates were put in the dark. After 20 min at room temperature the product formation was ended by addition of 100 µl/well of 2.5 M sulfuric acid. Following brief mixing, absorbance at 450 nm was measured. The inter-assay coefficient of variance was typically less than 10%, while the intra-assay coefficient of variance was typically less than 4%. Correlation coefficients of the calibration curves were typically better than 0.99. Reagent blanks had a typical absorbance of 0.06 (A450).

The following plates have relatively high mean values; 63,64,65,66,74,75, i.e. intra assay variance higher than 15%. We will analyze the data using all samples, in the first additional analysis we will exclude the abovementioned plates to see whether the results remain similar. Finally we will see if adjustment for these outlying plates is possible.