

### *Endogenous sex hormone levels*

Serum and plasma from the Year 3 visit were stored at  $-70^{\circ}\text{C}$  and sent directly from storage to the analytical laboratories (Royal Marsden, London, England, for estradiol and SHBG and Wake Forest University, NC, for testosterone) without thawing. Bioavailable plasma estradiol concentration was measured by radioimmunoassay using a highly specific rabbit antiserum raised against an estradiol-6-carboxymethyloximine-bovine serum albumin conjugate (EIR, Wurenlingen, Switzerland) and Third Generation Estradiol [I125] reagent (DSL 39120 Diagnostic Systems Laboratories Inc., TX). All samples were measured in duplicate. The lower detection limit is 0.8 pg/ml. Within-assay variability, assessed in 20 assays using 4 replicates of a serum pool, gave a mean estradiol level of 7.3 pg/ml and an overall within-assay coefficient of variation of 7.6%. The between-assay coefficient of variation (CV) for the same pool was 17% (N=20). The CV calculated using 5% blind duplicate samples was 41.5%. Part of the reason for the high CV, however, is that these results are highly skewed. After log transformation of the data, the CV was 11.2%.

Serum concentrations of free testosterone were measured using an enzyme immunoassay (EIA) kit (sensitivity = 0.19 pg/ml, detection range = 0.25-100 pg/ml) from Diagnostic Systems Laboratories, Inc. (DSL, Webster, TX). This kit utilizes a rabbit anti-testosterone anti-serum, which has low affinity for sex hormone binding globulin (SHBG) and albumin. All samples were measured in duplicate and the average of the two values was used for data analyses. The intra-assay and inter-assay coefficients of variation were 4.9 and 9.0%, respectively, for a low level control (1.9 pg/ml) and were 3.6 and 13.0%, respectively, for a high level control (26.6 pg/ml). The CV calculated using 5% blind duplicate samples was 62.2%, and after log transformation of the data was 41.4%.

SHBG was measured using the IMMULITE®, an automated continuous random-access chemiluminescent immunoassay (CLIA) system (Diagnostic Products Corporation, Los Angeles, CA). It had a detectable range of 1.0–34.7 pmol/l. The CV calculated using 5% blind duplicate samples was 36.2%, and after log transformation of the data was 7.3%.