Pre-study power analysis

A pre-study power analysis based on the BMD of the spine and the hip was performed. The mean BMD\textsubscript{spine} in healthy young women and TS has been found to be 0.98 \pm 0.10 g/cm\textsuperscript{2} and 0.87 \pm 0.11 g/cm\textsuperscript{2}, respectively, and mean BMD\textsubscript{hip} in healthy young women and TS has been found to be 0.83 \pm 0.08 g/cm\textsuperscript{2} and 0.68 \pm 0.07 g/cm\textsuperscript{2}, respectively (19), after pubertal induction using conventional hormonal replacement treatment. Expecting a correlation between the dose of 17\beta-estradiol, BMD\textsubscript{spine} (effect size 1.1) and BMD\textsubscript{hip} (effect size 2.0) in the TS population and applying the value of \(\alpha=0.05\) and \((1-\beta) = 0.70\), a number of between 4 (BMD\textsubscript{hip}) and 10 (BMD\textsubscript{spine}) TS patients in each treatment group would be sufficient to show a difference in BMD.

Safety

Side-effects and adverse events were closely monitored. Only one participant reported side-effects in terms of moderate oedema of the lower limbs and decided to leave the study. No adverse events were reported. A physical examination including height, weight, peripheral oedema, lung and heart auscultation, palpation of the abdomen, blood pressure, breast examination, and evaluation of endometrial hyperplasia by trans-abdominal ultrasound was performed at the baseline and the yearly visits. Safety parameters (creatinine, electrolytes, liver parameters, lipids, hemoglobin A1c, fasting blood glucose, thyroid hormones, hemoglobin, and C - reactive protein) were recorded at the baseline visit and the yearly visit. These were all within normative ranges and did not change during the course of the study.

Assays

As a marker of bone resorption plasma carboxy-terminal collagen crosslinks (CTX) were measured by electrochemiluminescence immunoassay. Markers of bone formation were plasma bone-specific alkaline phosphatase (BALP) measured by enzyme immunoassay and plasma Pro-collagen I amino-terminal pro-peptide (PINP) analyzed by electrochemiluminescence immunoassays.
All analyses of sex-hormones were performed at Statens Serum Institute. Androstendione, 17β-estradiol, estrone, estrone sulfate, dihydrotestosterone (DHT), 17OH-progesterone was measured by liquid-chromatography mass-spectrometry (LC-MS) using an accredited method. The LC-MS system consisted of a Surveyor quaternary pump (Thermo Fisher Scientific, Walram, MA), a CTC autosampler (CTC Analytics AG, Zwingen, Switzerland) and a TSQ ultra triple quadruple mass spectrometer (Thermo Fischer Scientific). Chromatographic separation was achieved using a hypersil GOLD C18 column (Thermo Fisher Scientific, 10 x 2, 1 mm, cm 3µm). The analysts were detected by tandem MS using selective reaction monitoring.

Quantification of LH and FSH levels: LH and FSH were quantified separately on the Kryptor® immunoassay analyser (Brahms, Hennigsdorf, Germany) using Time Resolved Amplified Cryptate Emission (TRACE) technology according to the manufacturer’s instructions. In brief, patient samples were incubated with either anti-LH or anti-FSH antibodies conjugated to either europium Cryptate (donor) or XL665 (acceptor). Upon excitation, energy was transferred from the donor to the acceptor antibody within the immuno-complex, thereby increasing and prolonging XL665 mediated emission and facilitating time-resolved fluorometry. The intensity of the fluorescence signal was directly proportional to the concentration of LH or FSH in the sample.

Quantification of SHBG levels: SHBG was quantified using a solid phase, two-site fluoroimmunometric assay on the autoDELFIA® 1235 immunoassay analyser (PerkinElmer, Waltham, MA) according to the manufacturer's instructions. In brief, patient samples were incubated with anti-SHBG antibodies immobilized in microtiter plates followed by incubation with europium-conjugated anti-SHBG antibodies. Following release of the europium-label from the immunocomplex, the fluorophores were exposed to pulsed excitation and quantified using time-resolved immuno-fluorometry. The intensity of the fluorescence signal was directly proportional to the SHBG concentration in the sample.