Materials and Methods

Study subjects: young adult men
Study subjects were randomly identified using national population registers, contacted by telephone, and asked to participate in the present study. To be included in the GOOD study, subjects had to be more than 18 and less than 20 yr of age and willing to participate in the study. Almost half (48.6%) of the study candidates agreed to participate and were enrolled (1). Serum sex steroid levels analyzed by the GC-MS technique (requiring 1 ml of serum) were available for 1060 (99%) of the study subjects. The study was approved by the ethics committee at the Göteborg University. Informed consent was obtained from all study participants.

Study subjects: elderly men, MrOS Sweden
To be eligible for the MrOS Sweden study, subjects had to be able to walk without aids. 45 % of those who were invited agreed to participate in the study. Serum sex steroid levels analyzed by the GC-MS technique were available for 2640 (88%) of the study subjects. The study was approved by the ethics committees at the Göteborg, Lund and Uppsala Universities. Informed consent was obtained from all study participants.

Study subjects: elderly men, MrOS US
Participants in the MrOS US study were intended, to the extent possible with a volunteer cohort, to be representative of the population of older men in the communities from which they were recruited. Eligibility criteria were 1) ability to walk without the assistance of another, 2) absence of bilateral hip replacements, 3) ability to provide self-reported data, 4) anticipated residence near a study site for the duration of follow-up, 5) absence of a medical condition that would result in imminent death, and 6) ability to understand and sign an informed consent. MrOS participants whose serum was used for the measurement of sex steroids were selected using a stratified sampling design. The two strata were non-Hispanic white and Hispanic, or non-white. Within the non-Hispanic white stratum, participants were sampled with a probability of 27% (n=1439). All Hispanic or non-white participants were sampled (n=609). Institutional Review Boards approved the study protocol, and written informed consent was obtained from all participants.

Assessment of covariates
Height was measured using a Harpender stadiometer. Weight was measured on a digital scale in all Swedish and in 1/6 US center, and on a balance bean scale in 5/6 US centers.
The proportion of current smokers was rather similar for the young adult men in the GOOD cohort (8.7%, (2)) and the elderly men in the MrOS Sweden cohort (8.5%, (3)).
In the MrOS Sweden cohort, the self reported physical activity was the subject`s average total daily walking distance in km, including both walking as a means of exercise and leisure, and as a means of outdoor transportation in activities of daily life (3). In the GOOD cohort, the self reported physical activity was the subject's present hours/week of physical activity (1).

Assessment of gonadotropins and SHBG
In the GOOD cohort FSH was measured using IRMA (Diagnostic Systems Laboratories, Texas, USA), limit of detection 0.11 mIU/ml, intra-assay CV 3.5%, inter assay CV 6.2%, while LH was measured using ELISA (Diagnostic Systems Laboratories), limit of detection 0.10 mIU/ml, intra-assay CV 5.6%, inter assay CV 7.6%.
In the GOOD and MrOS Sweden cohorts serum SHBG was measured using IRMA (Orion Diagnostics), limit of detection 1.3 nmol/l, intra-assay CV 3 %, inter assay CV 7 %. In the MrOS US cohort serum SHBG was measured using an Immulite Analyzer with chemiluminescent substrate (Diagnostic Products Corp., Los Angeles, California, USA), limit of detection 0.2 nmol/l, intra-assay CV 5.6 %, inter-assay CV: 6.5 %.

Assessment of sex steroids in the MrOS US cohort
Briefly, the analytes and their deuterated internal standards were extracted from 1.00 mL of human serum using BondElut Certify® solid phase cartridges. Estradiol, Estrone, and Testosterone were

eluted from the solid phase cartridges with ethyl acetate. The analytes underwent three separate derivatization steps: 1) reaction with pentafluorobenzoyl chloride, 2) reaction with O (2,3,4,5,6 pentafluorobenzyl) hydroxylamine hydrochloride, and 3) reaction with N-Methyl-N-(trimethylsilyl)trifluoroacetamide. Then the derivatized analytes were separated by gas chromatography using a DB 17 fused silica capillary column and detected by tandem mass spectrometry using negative ion chemical ionization,

Genotyping
MrOS Sweden: The SNP rs2470152 in CYP19 and the SNP rs4953616 in LHCGR were analyzed using matrix-assisted laser desorption ionization-time of flight mass spectrometry on the Sequenom MassARRAY platform (San Diego, CA, USA). The genotyping call rate was 97.3% for rs2470152 and 96.9 % for rs4953616. Both SNP-s were in Hardy-Weinberg equilibrium.
MrOS US: The SNP rs2470152 in CYP19 was analyzed using the TaqMan Assay on Demand™ (Applied Biosystems, Foster City, CA, USA). The genotyping call rate was 96.3% and the genotyping reproducibility was 99.7%. The SNP rs2470152 was in Hardy-Weinberg equilibrium.

Assessments of bone mineral density and self reported previous fractures in GOOD and MrOS Sweden
Bone mineral density (BMD) of the lumbar spine and femoral neck were measured using dual energy X-ray absorptiometry (DXA). In the GOOD cohort the Lunar Prodigy DXA (GE Lunar Corp., Madison, WI USA) was used (4). In the MrOS Sweden cohort, a Hologic QDR 4500 / A-Delphi (QDR 4500 W, Hologic, Inc.) was used in Gothenburg and a Lunar Prodigy DXA (GE Lunar Corp.) in Malmö and Uppsala (3). To allow pooling of DXA measurements performed with different equipments (Lunar Prodigy and Hologic QDR 4500 / A-Delphi) in the MrOS Sweden cohort, standardized BMD (sBMD) was calculated using previously reported algorithms (5-7). BMD was adjusted for age, body mass index (BMI), smoking and physical activity.
Self-reported information on previous fractures was obtained using standardized questionnaires. At inclusion, 29.2% (298/1022) of the GOOD subjects with available rs2470152 genotyping and available information on previous fractures had at least one self reported fracture (8). 17.4% (502/2889) of the MrOS Sweden subjects with available rs2470152 genotyping and available information on fractures had at least one self reported fracture after 50 years of age (3).

REFERENCES

1. **Lorentzon M, Mellstrom D, Ohlsson C** 2005 Age of attainment of peak bone mass is site specific in Swedish men--The GOOD study. J Bone Miner Res 20:1223-1227
2. **Lorentzon M, Mellstrom D, Haug E, Ohlsson C** 2007 Smoking is associated with lower bone mineral density and reduced cortical thickness in young men. The Journal of clinical endocrinology and metabolism 92:497-503
3. **Mellstrom D, Johnell O, Ljunggren O, Eriksson A, Lorentzon M, Mallmin H, Holmberg A, Redlund-Johnell I, Orwoll E, Ohlsson C** 2006 Free Testosterone is an Independent Predictor of BMD and Prevalent Fractures in Elderly Men - MrOs Sweden. J Bone Miner Res 21:529-535
4. **Lorentzon M, Swanson C, Andersson N, Mellstrom D, Ohlsson C** 2005 Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. J Bone Miner Res 20:1334-1341
5. **Hui SL, Gao S, Zhou XH, Johnston CC, Jr., Lu Y, Gluer CC, Grampp S, Genant H** 1997 Universal standardization of bone density measurements: a method with optimal properties for calibration among several instruments. J Bone Miner Res 12:1463-1470
6. **Lu Y, Fuerst T, Hui S, Genant HK** 2001 Standardization of bone mineral density at femoral neck, trochanter and Ward's triangle. Osteoporos Int 12:438-444
7. 1995 Standardization of spine BMD measurements. J Bone Miner Res 10:1602-1603
8. **Kindblom JM, Lorentzon M, Norjavaara E, Hellqvist A, Nilsson S, Mellstrom D, Ohlsson C** 2006 Pubertal timing predicts previous fractures and BMD in young adult men: the GOOD study. J Bone Miner Res 21:790-795