

CLINICAL STUDY

Secular trends in sex hormones and fractures in men and women

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Abstract

Objective: To study secular trends in sex hormones, anthropometry, bone measures and fractures.

Design: A random population sample was studied twice and subjects of similar age group were compared 13 years apart.

Methods: X-ray-verified fractures were retrieved from a random population sample of 2400 men and women (participants 1616 = 67%) aged 25–64 years from the WHO, MONICA Project in Gothenburg, Sweden, in 1995 and 2008. Fasting serum hormones and calcaneal ultrasound were measured in every fourth subject. In fertile women, measurements were performed on cycle day interval 7–9.

Results: In 2008, men had lower serum free testosterone than men of similar age in 1995 ($P < 0.001$). Body composition, physical activity and fracture incidence were similar. In women, hormone replacement therapy (HRT) was lower in 2008, 7 vs 28% ($P < 0.0001$), as was serum oestradiol, although use of tranquilisers and leisure time physical activity were higher. In 2008, the fracture incidence was higher in postmenopausal women, 29 vs 17% ($P < 0.001$), and vertebral crush had increased from 8 to 19% of all fractures ($P = 0.031$). Serum cholesterol and triglycerides were lower in all subjects in 2008 compared with that in 1995.

Conclusions: Secular trends were observed with lower serum testosterone in men in 2008, but no effect was seen on the fracture incidence of these fairly young men. In postmenopausal women in 2008, there was a higher fracture incidence along with more vertebral compressions. Lower HRT use, lower serum oestradiol and higher fall risk exposure due with more tranquilisers and leisure time physical activity in 2008 may explain the results.

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Introduction

Circulating sex hormone concentrations decrease with increasing age in men and women, and this is accentuated in women during the menopause along with osteoporosis and fractures. During the past few decades, hormone replacement therapy (HRT) has largely been used as a prophylactic measure. However, in the mid-1990s, HRT prescriptions almost ceased due to the Women's Health Initiative report on the increased risk of cancer (1).

A gradual decrease in testosterone in men around 50 years of age has also been reported (2, 3). However, there are no clear clinical signs of gonadal insufficiency in men compared with women who stop menstruating and lose their fertility at that time. A prospective study showed an age-independent decline in serum testosterone in American men aged 45–79 years during 17 years of follow-up, which is indicative of secular trends like environmental factors influencing the

gonads (4). A recent Danish study also reported similar declines (5). Another report showed earlier onset of puberty during 15 years of follow-up in Danish boys, which was explained by an increase in body weight (6). Travison *et al.* (7) claimed in a review that adiposity had a substantial contributory role for the age-independent decline in testosterone in these studies on adult men from the USA and Europe (4, 5). They stated that further confirmatory and more explanatory population studies were needed.

This study had two aims. The first aim was to determine whether the decline in serum testosterone in American and Danish men was also observed in the Swedish population by analysing a subsample of a random population sample examined in 1995 and 2008. The age of the compared participants was the same in 2008 as in 1995. Body composition, lifestyle factors and pharmacological treatment were compared 13 years apart. The second aim was to analyse the incidence of fractures and, for this, all 1616 participants

of the random samples from 1995 and 2008 were available. Associated with this aim was an analysis of fractures in relation to sex hormones, lifestyle factors, bone measurements and HRT (in women) in the subsample for which these measurements were available.

Materials and methods

Subjects

A random population sample of 2400 men and women (participants 1616) aged 25–64 was recruited from the city census, which is kept up to date within a maximum of 14 days, in 1995. This was the third population screening by the World Health Organization (WHO), Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA), Gothenburg, Sweden (8). The non-attenders could not participate due to travelling, living abroad, unwillingness, or being incapable of attending due to illness of a relative.

Hormone and bone measurements were performed in every fourth subject in the age groups 25–34, 35–44, 45–54, and 55–64 in men, and 25–34 and 35–44 in women, and all women aged 45–64. This latter group is denoted as postmenopausal in this study. Menstruation and age at menopause (the last bleeding) were asked for and serum FSH was analysed. A serum FSH > 50 U/l was considered postmenopausal according to the Laboratory for Clinical Chemistry at the hospital. Median S-FSH was 5, 64 and 59 U/l respectively in women aged 35–44, 45–54 and 55–64 years both in 1995 and 2008. One woman in the youngest age group was postmenopausal in 2008 and six women in the two oldest age groups were still menstruating in 2008 and were included in the respective group regarding fracture count.

The subjects were invited for re-evaluation in 2008 after a mean of 13 years of follow-up. Men and women of similar age 13 years apart were compared. Fifty-one subjects had died or could not be traced. The remaining numbers are shown in Tables 1 and 2 containing hormonal data for 581 randomly selected subjects in 1995 (151 men and 430 women) and 210 subjects (61 men and 149 women) in 2008 aged 35–44, 45–54 and 55–64 years.

Fracture records were derived from the Gothenburg hospital register via the National Board of Health and Welfare, Stockholm, Sweden, on 1616 subjects out of 1275 in the age group 35–64 years in 1995 and 1165 in the same age group in 2008 (Table 3 presents a study of possible secular trends in fracture incidence).

Each participant was given written information about the aim of the study, which was approved by the ethics committee of the University of Gothenburg and the National Data Inspection Board, and all participants provided written informed consent.

Study design

A random population sample, 25–64 years, was studied twice and subjects of similar age group, 35–64 years, were compared 13 years apart. Because of the 13-year increase in age by 2008, the youngest age group (25–34 years) in 1995 was not available in 2008, and for the oldest men and women (>65 years) in 2008, there were no counterparts in 1995. For the other age groups, the number of participants was lower in 2008 because of non-attendance.

Anthropometry

Body weight was measured once to the nearest 0.1 kg in the fasting state with the subject in underwear and without shoes. Body height was measured once to the nearest 1.0 cm. The body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2). The waist circumference was measured with a soft tape midway between the lowest rib margin and the iliac crest in the standing position. The hip circumference was measured over the widest part of the gluteal region and the waist/hip circumference ratio (WHR) was calculated. A single operator performed the measurements on both occasions.

Bioimpedance and bone measurement

Fat-free mass (FFM) and body fat were estimated using impedance measurements (SEAC Multiple frequency bioimpedance meter model SFB 2, UniQuest Ltd, Queensland, St. Lucia, Australia), based on total body resistance and reactance. Calcaneal Quantitative UltraSound (QUS) (LUNAR Achilles; General Electric Healthcare, Madison, WI, USA) was performed and has been described in detail previously (9).

Lifestyle factors

Past and present health status, smoking habits graded 1–3 (current smokers, ex-smokers and non-smokers), coffee consumption (cups/day), medication and physical activity during work and leisure time, graded 1–4 (low–high), were assessed with a validated questionnaire (10) which was self-administered before the physical examination.

Pharmacological treatment

Ongoing pharmacological treatment was asked after with similar questionnaires, both in 1995 and 2008, and was coded according to the Anatomical Therapeutic Chemical classification system. Tranquillizers, sedatives, antidepressants and central nervous system-acting analgesics were included in the N-group.

Table 1 Body composition, calcaneal ultrasound measurements, physical activity during leisure time and at work (graded 1–4, low-high), smoking, coffee consumption, blood lipids, IGF1, sex hormones and 10-year fracture probability (FRAX) in men of three different age groups in 1995 and 2008. Data are presented as means (s.d.) or as indicated.

Variables	Age groups					
	35–44		45–54		55–64	
	1995	2008	1995	2008	1995	2008
<i>n</i>	49	6	47	26	55	29
Age (years)	40.0 (2.6)	41.5 (2.3)	49.9 (2.9)	49.7 (2.6)	60.0 (2.7)	59.4 (2.8)
Height (cm)	178.0 (8.1)	180.3 (6.1)	177.0 (8.0)	178.1 (7.1)	177.4 (4.9)	179.2 (7.7)
Weight (kg)	81.9 (15.9)	82.2 (10.1)	81.9 (13.5)	84.0 (12.5)	83.7 (13.2)	90.3 (13.8)*
BMI (kg/m ²)	25.9 (5.1)	25.3 (2.6)	26.1 (3.5)	26.5 (3.6)	26.6 (3.8)	28.1 (3.7)
WHR	0.91 (0.05)	0.80 (0.15)*	0.93 (0.05)	0.91 (0.06)	0.96 (0.05)	0.97 (0.07)
FFM (kg)	62.9 (8.6)	67.2 (9.8)	62.7 (9.4)	64.2 (7.0)	62.6 (8.4)	64.2 (6.7)
Fat (kg)	22.5 (6.1)	15.1 (7.8)*	23.0 (5.9)	19.9 (8.7)*	24.5 (5.7)	26.1 (9.4)
SOS (m/s)	1537 (38)	1557 (57)	1531 (29)	1520 (29)	1517 (27)	1522 (34)
BUA (db/mHz)	114.9 (13.2)	123.0 (11.5)	113.4 (9.5)	112.3 (10.5)	111.0 (9.1)	114.7 (10.9)
Stiffness (%)	87.6 (17.7)	99.0 (24.1)	84.3 (13.0)	80.5 (14.2)	78.7 (13.2)	83.8 (16.0)
Physical activity leisure (<i>n</i> (%))						
1 Low	14 (28.6)	0 (0)	9 (19.1)	6 (23.1)	13 (23.6)	5 (17.9)
2	23 (46.9)	2 (33.3)	31 (66.0)	8 (30.8)	38 (69.1)	17 (60.7)
3	12 (24.5)	4 (66.7)	7 (14.9)	12 (46.2)	4 (7.3)	5 (17.9)
4 High	0 (0)	0 (0)*	0 (0)	0 (0)	0 (0)	1 (3.6)
Physical activity work (<i>n</i> (%))						
1 Low	15 (30.6)	4 (66.7)	16 (36.4)	13 (50.0)	18 (36.0)	10 (38.5)
2	16 (32.7)	2 (33.3)	16 (36.4)	8 (30.8)	18 (36.0)	8 (30.8)
3	11 (22.4)	0 (0)	8 (18.2)	4 (15.4)	11 (22.0)	5 (19.2)
4 High	7 (14.3)	0 (0)	4 (9.1)	1 (3.8)	3 (6.0)	3 (11.5)
Smoking (<i>n</i> (%))						
1 Current smokers	6 (12.8)	0 (0)	14 (29.8)	4 (16.0)	12 (22.2)	4 (14.3)
2 Former smokers	12 (25.5)	2 (33.3)	14 (29.8)	9 (36)	24 (44.4)	12 (42.9)
3 Non-smokers	29 (61.7)	4 (66.7)	19 (40.4)	12 (48.0)	18 (33.3)	12 (42.9)
Coffee (cups/day)	3.92 (2.53)	3.83 (2.23)	4.16 (2.52)	3.42 (2.08)	5.58 (6.10)	3.81 (1.86)
S-triglycerides (mmol/l)	1.98 (1.75)	1.03 (0.48)	2.13 (1.59)	1.23 (0.60) [†]	2.29 (2.15)	1.35 (0.67) [‡]
S-cholesterol (mmol/l)	5.66 (1.24)	5.32 (0.37)	6.23 (1.38)	5.35 (0.95) [†]	6.37 (1.21)	5.41 (1.14) [‡]
S-HDL (mmol/l)	1.29 (0.32)	1.60 (0.36)	1.24 (0.34)	1.48 (0.37) [†]	1.26 (0.44)	1.36 (0.33)
S-IGF1 (μg/l)	173.3 (51.0)	144.0 (13.5)	146.0 (42.5)	131.6 (38.9)	132.9 (42.4)	117.6 (35.9)
S-SHBG (nmol/l)	28.8 (9.3)	32.6 (8.1)	33.3 (15.8)	39.0 (16.6)	44.0 (43.7)	38.1 (13.3)
S-oestradiol (pmol/l)	0.079 (0.027)	0.078 (0.025)	0.078 (0.023)	0.078 (0.020)	0.083 (0.027)	0.070 (0.016)
S-testosterone (nmol/l)	20.2 (4.2)	16.9 (2.7)	19.8 (4.3)	18.2 (3.7)	17.0 (5.5)	13.6 (3.3) [‡]
S-free testosterone (nmol/l)	0.48 (0.12)	0.37 (0.04)*	0.44 (0.11)	0.34 (0.11) [‡]	0.34 (0.11)	0.27 (0.07) [‡]
FRAX, major fracture risk (%)	1.66 (0.73)	1.56 (0.67)	3.73 (1.49)	5.03 (1.9)*	6.56 (2.59)	6.72 (2.76)
FRAX, hip fracture risk (%)	0.13 (0.15)	0.1 (0.1)	0.38 (0.36)	0.74 (0.73)*	1.72 (0.82)	1.38 (0.82)

* $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$ for comparison between groups of similar age. WHR, waist/hip ratio; FFM, fat-free mass; SOS, speed of sound; BUA, broadband ultrasound attenuation; SHBG, sex hormone-binding globulin.

Blood samples

After an overnight fast, venous blood samples were collected (in menstruating women, according to the interview, on cycle day 7–9) and analysed within 1 year.

Concentrations of serum total cholesterol, HDL cholesterol and triglycerides were determined enzymatically (Boehringer).

Serum total testosterone was determined by non-extraction competitive RIA (ICN Biochemicals, Inc. Diagnostics Division, Costa Mesa, CA, USA). The coefficient of variation (CV) for total testosterone levels was 16.3% at 2.0 nmol/l and 10.0% at 26.8 nmol/l. Serum total oestradiol was determined by RIA (Clinical Assays Estradiol-2, DiaSorin, Saluggia, Italy). The CV for oestradiol levels was 10.0% at 0.4 pmol/l and 16.0% at 0.04 pmol/l. Sex hormone-binding globulin (SHBG)

was determined by IRMA (Orion Diagnostica Oy, Espoo, Finland). The CV for SHBG levels was 4.2% at 19.7 nmol/l and 6.3% at 76.3 nmol/l. Serum free testosterone was calculated according to Vermeulen *et al.* (11) and based on that, concentrations of total testosterone = free testosterone + albumin-bound testosterone + SHBG-bound testosterone. The tool for calculating free testosterone is provided by the International Society for the Study of the Aging Male (ISSAM) on the internet at <http://www.issam.ch/freetesto.htm>. Serum IGF1 was determined by RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) in 1995. The CV for IGF1 was 8% at levels of 67 μg/l and 6% at 332 μg/l. In this study, the Siemens Immulite 2500 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA), which was 28% lower than the Nichols RIA, was used. The equation used was $y = 1995 \text{ value} \times 0.7245 +$

Table 2 Body composition, calcaneal ultrasound measurements, physical activity during leisure time and at work (graded 1–4, low-high), smoking, coffee consumption, blood lipids, IGF1, sex hormones and FRAX in women of three different age groups in 1995 and 2008. Data are presented as means (s.d.) or as indicated.

Variables	Age group					
	35–44		45–54		55–64	
	1995	2008	1995	2008	1995	2008
<i>n</i>	71	14	184	39	175	96
Age (years)	40.9 (2.6)	41.5 (2.0)	50.7 (2.6)	50.4 (3.2)	60.5 (2.5)	60.7 (2.8)
Height (cm)	165.0 (6.4)	168.4 (5.3)	165.8 (6.4)	164.6 (7.3)	163.5 (5.9)	164.7 (6.3)
Weight (kg)	64.4 (10.2)	72.7 (11.3)*	69.2 (12.29)	67.6 (12.1)	70.2 (12.3)	72.0 (14.9)
BMI (kg/m ²)	23.6 (3.4)	25.5 (3.2)	25.2 (4.3)	24.9 (4.0)	26.3 (4.5)	26.6 (5.5)
WHR	0.79 (0.05)	0.80 (0.059)	0.80 (0.06)	0.83 (0.06) [†]	0.81 (0.06)	0.83 (0.07)*
FFM (kg)	47.0 (6.4)	49.3 (5.3)	48.1 (6.5)	45.2 (6.2) [†]	47.4 (6.2)	45.5 (5.7)*
Fat (kg)	27.0 (5.4)	23.4 (11.3)	29.8 (8.3)	22.5 (9.2) [‡]	31.4 (7.8)	26.5 (11.8) [‡]
SOS (m/s)	1533 (27)	1523 (33)	1526 (27)	1529 (29)	1510 (25)	1508 (29)
BUA (db/mHz)	110.1 (10.5)	113.9 (12.6)	107.7 (9.4)	112.4 (11.1) [†]	102.8 (10.7)	106.1 (9.3) [†]
Stiffness (%)	82.7 (13.2)	82.4 (16.8)	79.0 (12.8)	82.9 (14.1)	71.6 (10.9)	73.0 (13.5)
Physical activity leisure (<i>n</i> (%))						
1 Low	14 (19.7)	3 (21.4)	43 (23.4)	2 (5.3)	28 (16)	11 (11.6)
2	40 (56.3)	3 (21.4)	126 (68.5)	23 (60.5)	132 (75.4)	57 (60.0)
3	17 (23.9)	7 (50)	15 (8.2)	12 (31.6)	14 (8)	27 (28.4)
4 High	0 (0)	1 (7.1)	0 (0)	1 (2.6) [‡]	1 (0.6)	0 (0) [‡]
Physical activity work (<i>n</i> (%))						
1 Low	21 (30)	6 (42.9)	71 (40.3)	23 (63.9)	45 (30.6)	41 (47.7)
2	26 (37.1)	8 (57.1)	72 (40.9)	9 (25)	73 (49.7)	32 (37.2)
3	22 (31.4)	0 (0)	33 (18.8)	4 (11.1)	29 (19.7)	12 (14.0)
4 High	1 (1.4)	0 (0)*	0 (0)	0 (0)*	0 (0)	1 (1.2)*
Smoking (<i>n</i> (%))						
1 Current smokers	20 (28.6)	3 (21.4)	41 (22.4)	4 (10.8)	42 (24)	11 (11.5)
2 Former smokers	11 (15.7)	5 (35.7)	48 (26.2)	13 (35.1)	33 (18.9)	31 (32.3)
3 Non-smokers	39 (55.7)	6 (42.9)	94 (51.4)	20 (54.1)	100 (57.1)	54 (56.3)
Coffee (cups/day)	3.49 (2.34)	4.18 (3.09)	3.91 (2.32)	2.82 (1.78) [†]	3.47 (1.99)	3.09 (1.70)
S-triglycerides (mmol/l)	1.12 (0.48)	0.81 (0.33) [†]	1.47 (0.85)	0.96 (0.53) [‡]	1.53 (0.82)	1.18 (0.62) [‡]
S-cholesterol (mmol/l)	5.52 (0.91)	4.80 (0.77) [†]	6.01 (1.21)	5.19 (0.77) [‡]	6.47 (1.02)	5.71 (1.09) [‡]
S-HDL (mmol/l)	1.58 (0.45)	1.67 (0.41)	1.59 (0.40)	1.83 (0.49) [†]	1.62 (0.40)	1.83 (0.40) [‡]
S-IGF1 (μg/l)	174.8 (56.9)	158.9 (30.0)	151.9 (53.8)	123.4 (39.4)*	118.4 (31.9)	112.1 (35.8)
S-SHBG (nmol/l)	59.2 (27.2)	56.4 (16.8)	60.4 (32.2)	57.1 (23.5)	58.6 (27.2)	59.7 (28.7)
S-oestradiol (pmol/l)	0.383 (0.381)	0.370 (0.226)	0.397 (0.608)	0.200 (0.244)*	0.073 (0.096)	0.072 (0.184)
S-testosterone (nmol/l)	1.06 (0.49)	0.72 (0.31) [†]	0.90 (0.61)	0.65 (0.29)	0.76 (0.350)	0.70 (0.49)
S-free testosterone (nmol/l)	0.015 (0.008)	0.010 (0.006)	0.012 (0.009)	0.009 (0.005)	0.011 (0.007)	0.010 (0.007)
FRAX, major fracture risk (%)	1.48 (0.49)	1.58 (0.64)	4.69 (1.67)	6.52 (2.46) [†]	8.23 (3.04)	10.63 (4.23) [†]
FRAX, hip fracture risk (%)	0.15 (0.16)	0.16 (0.13)	0.65 (0.60)	1.11 (0.79) [‡]	1.74 (1.2)	2.42 (1.72) [†]

* $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$ for comparison between groups of similar age. WHR, waist/hip ratio; FFM, fat-free mass; SOS, speed of sound; BUA, broadband ultrasound attenuation; SHBG, sex hormone-binding globulin.

12.6245, $r = 0.9859$ ($n = 138$). Only converted values are shown.

Fractures

Records of X-ray-verified fractures deemed to be of osteoporotic origin (upper arm, wrist, ankle, leg, hip, pelvis, rib and vertebrae), according to ICD 10 codes S22, S32, S42, S52, S62, S72, S82, S92, T08, T10, T12 and T14, were retrieved from the Gothenburg hospital registers via the National Board of Health and Welfare, Stockholm, Sweden. Fractures related to accidents were not included. Only the first fracture was included in the analysis. Ten-year probability for major osteoporotic and hip fracture respectively, based on FRAX, was calculated on all examined subjects in Tables 1 and 2.

This is an assessment tool based on current age, BMI, parental hip fracture, previous fracture, corticosteroid use, rheumatoid arthritis, other secondary osteoporosis, smoking and alcohol consumption provided by <http://www.shef.ac.uk/FRAX/index.htm> (12).

Statistical analysis

Medians, means and s.d. were calculated using conventional methods. For comparison between groups, the Mantel–Haenszel χ^2 test was used for ordered categorical variables and the Mann–Whitney U test for continuous variables.

A P value of < 0.05 (two-sided test) was considered statistically significant. Odds ratios (OR) were calculated using the χ^2 test for the analysis of proportions between

Table 3 Number (%) of subjects with fractures in men and women of different age groups, 13 years apart, in 1995 and 2008.

	Age groups					
	35–44		45–54		55–64	
	1995	2008	1995	2008	1995	2008
Men						
Subjects (n)	185	151	189	185	216	189
Fractures (n)	30 (16.2%)	15 (9.9%)	32 (16.9%)	19 (10.3%)	32 (14.8%)	33 (17.5%)
Women						
Subjects (n)	220	178	242	220	223	242
Fractures (n)	18 (8.9%)	28 (15.7%)*	33 (13.6%)	50 (22.7%) [†]	45 (20.2%)	85 (35.1%) [‡]

* $P < 0.05$, [†] $P < 0.01$, [‡] $P < 0.001$ for comparison between groups of similar age.

groups. We are aware of the fact that the 2008 groups are not independent of those of 1995, which may influence the test used.

Comparisons were made between subjects within the same age groups in 1995 and 2008. Due to the second investigation being conducted 13 years later, we could not compare subjects in the youngest age group of 25–34 in 1995, or the oldest, who reached ages above 65 years in 2008. In the age group 35–44, the numbers were only six men and 14 women, making the reports less conclusive.

Results

Anthropometry, lifestyle factors and hormones in men are given in Table 1.

In the 35–44-year-old men of 2008, serum free testosterone was lower compared with men of the same age in 1995 (Table 1 and Fig. 1a and b), and the WHR and body fat were also lower in 2008. Physical activity at leisure time was higher and at work tended to be lower in 2008. There were fewer men in 2008 for which reason these figures should be considered with great caution.

In the 45–54-year-old men of 2008, serum free testosterone, body fat, total cholesterol and triglycerides were lower than that of the men of similar age in 1995 (Table 1 and Fig. 1a and b). HDL cholesterol level in the male subjects of 2008 was higher than that of 1995.

In the 55–64-year-old men of 2008, serum total and free testosterone as well as total cholesterol and triglycerides were lower than that of the men of similar age in 1995. Body weight was higher (Table 1 and Fig. 1a and b). According to this study and other studies (13, 14, 15), there is a negative correlation between testosterone and body weight. Thus, serum total testosterone values in men aged 55–64 years were corrected for body weight, but weight-corrected testosterone was still significantly lower in 2008 than in 1995 ($P = 0.0384$).

Anthropometry, lifestyle factors and hormones in women are given in Table 2.

In the 35–44-year-old women of 2008, body weight was higher, but blood lipids, total testosterone ($P = 0.014$) and HRT use (mainly oral contraceptives) were lower than that of the women of similar age in 1995 (Table 2 and Fig. 2a).

In the 45–54-year-old women of 2008, WHR was higher, but IGF1, serum oestradiol ($P = 0.048$), FFM and body fat were lower compared with the women of the same age in 1995. QUS, HDL and physical activity during leisure time were higher, whereas total cholesterol, triglycerides and physical activity at work were lower in 2008 (Table 2). HRT use was lower in 2008, 4 vs 34%, OR 0.09 (CI 0.01–0.37, $P = 0.00008$), Fig. 2a. Serum oestradiol in women without HRT in 1995 was 0.258 pmol/l and did not differ from those in 2008 without HRT. Serum testosterone did not differ between women with and without HRT in 1995 or 2008.

In the 55–64-year-old women of 2008, WHR, HDL, QUS and physical activity during leisure time were higher, while FFM, body fat, physical activity at work, total cholesterol and triglycerides were lower compared with women of similar age in 1995. In 2008, HRT use was lower, 10 vs 37%, OR 0.18 (CI 0.09–0.36, $P < 0.00001$). Serum oestradiol or testosterone did not differ between those with and without HRT use in 1995 or 2008.

Results of the second aim: fractures

The number of fractures was similar in the compared age groups of men in 1995 and in 2008. In the 55–64-year-old men, the fracture incidence was slightly higher (ns) in 2008, OR 1.54 (CI 0.98–2.42, $P = 0.050$), Table 3.

In the 35–44-year-old women, the fracture incidence was higher in 2008 compared with 1995; OR 2.09 (CI 1.07–4.17, $P = 0.019$), Table 3 and Fig. 2b. Among women aged 45–54, fractures were more common in 2008 than in 1995, 22.7 vs 13.6%, OR 1.86 (CI 1.12–3.13, $P = 0.011$). Fractures were also more common in the age group 55–64 in 2008, with an increase from 20.2 to 35.1%, OR 2.14 (CI 1.38–3.34, $P = 0.00034$).

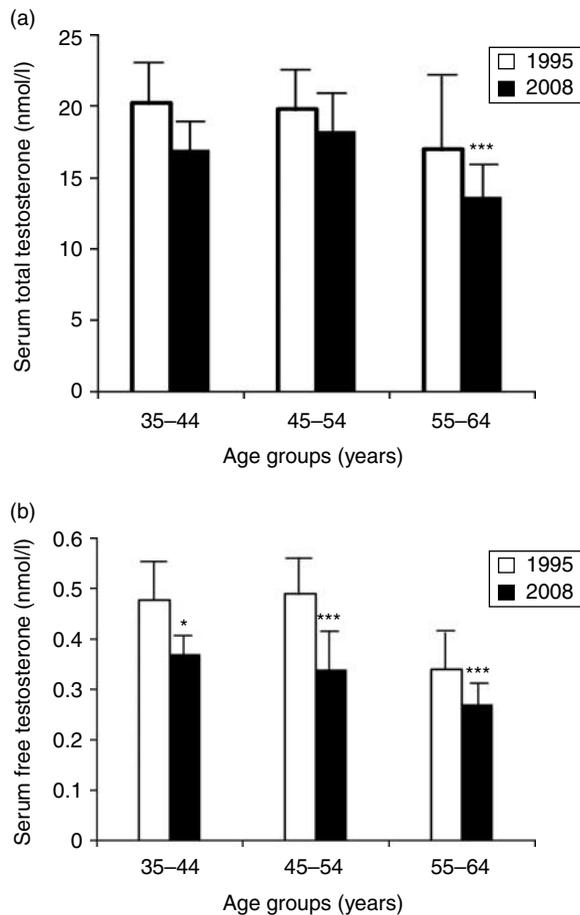


Figure 1 Serum total testosterone (a) and serum free testosterone (b) in men in 1995 (white bars) and in 2008 (black bars) for similar age groups 13 years apart. Means \pm s.d. are given. * $P < 0.05$, *** $P < 0.001$.

The total number of fractured women in 2008 was almost twice as high; 26 vs 14% of all women in 1995 in similar ages, OR 2.25 (CI 1.49–3.36, $P = 0.00003$). In 1995, more women in general used HRT. Of all women with fractures in 1995, only 12% were on HRT whereas 31% of the non-fractured women had HRT; OR 0.31 (CI 0.14–0.60, $P = 0.00022$). However, in 2008, mainly women with fractures used HRT; 18 vs only 3.5% of non-fractured women used HRT, OR 5.91 (CI: 1.65–23.48, $P = 0.0008$).

Postmenopausal women in the 45–64-year age group of 2008 ($n = 457$) had more fractures (29.2 vs 16.9%, OR = 2.05 (CI 1.48–2.85, $P = 0.0000067$)) than women of the corresponding age group in 1995 ($n = 465$), Fig. 2b. Of all fractures, vertebral crush accounted for 7.7% in 1995 and 18.9% in 2008, OR = 2.73 (CI 1.02–8.50, $P = 0.031$), Fig. 2b. In 2008, 17% had fractured once, 8% had fractured twice and 4% had fractured thrice or more, compared with 11, 4 and 3% respectively in the 1995 cohort. HRT use among postmenopausal women was lower in 2008, 8

vs 35%, OR = 0.17 (CI 0.09–0.30, $P < 0.0001$), compared with their counterparts in 1995 (Fig. 2a). FRAX percentage was higher in postmenopausal women in 2008 compared with those in 1995 (Table 2).

Other factors affecting osteoporosis

Fifteen percent of all men and women and 3.5% of postmenopausal women, respectively, in the 2008 cohort received lipid-lowering or anti-osteoporotic agents but no subject used these drugs in 1995. Calcium/vitamin D supplementation/treatment due to osteoporosis was used by 10% in 2008 and 0.5% in 1995. The number of antihypertensive agents was similar in 2008 and 1995. Tranquilisers (N-group in ACT) were similarly used in men in 2008 (7, 12 and 20% for age groups 35–44, 45–54 and 55–64) as in men in 1995 (7, 10 and 13%). Tranquiliser use was higher in women of 2008 (42, 24 and 28%) versus 1995 (14, 16 and 17%), OR = 1.58 (CI 1.09–2.30, $P < 0.01$).

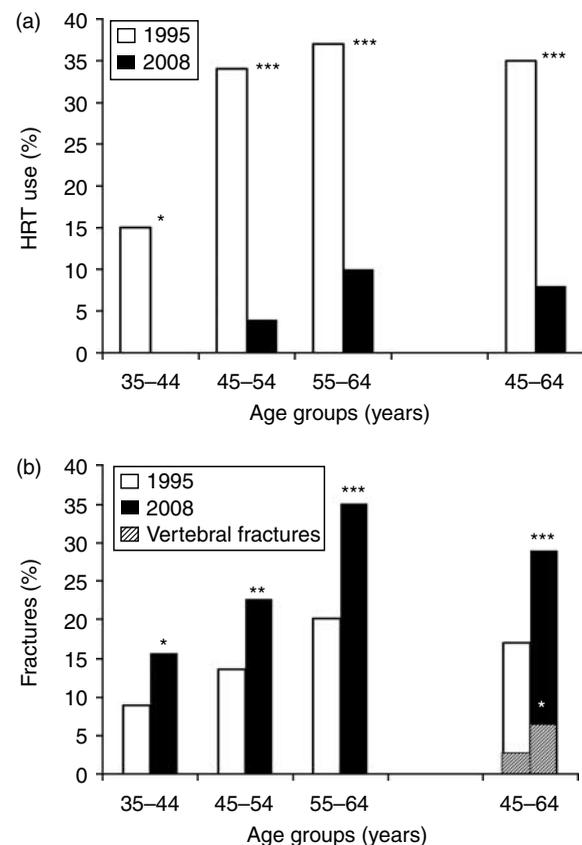


Figure 2 Oestrogen hormone replacement (HRT) use (a) and number (%) of subjects with fractures (b) in women 35–64 years and in postmenopausal women 45–64 years in 1995 (white bars), compared with similar age groups in 2008, 13 years apart (black bars). Striped bars in postmenopausal women show the percentage of vertebral compression (8% and 19% of all fractures in 1995 and 2008 respectively). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Smoking was lower in 2008, 11 vs 26% in the whole cohort of 1995. One man in the age group 55–64 in 1995 was operated due to prostate cancer and was excluded from the fracture count. None had been diagnosed with prostate cancer in 2008.

Discussion

Serum free testosterone was lower in 2008 compared with 1995 among all men, and total testosterone was lower in men aged 55–64 years. The secular trends in men confirm a previous American study showing an age-independent decline in serum testosterone in men aged 45–79 years during 17 years of follow-up (4). A Danish study showed similar secular trends in men of corresponding ages as the present study (5).

The 45–54-year-old men in 2008 had the same body weight and a more favourable serum lipid profile than that of the men of the same age 13 years earlier. Hence, the lower free testosterone concentrations in 2008 cannot be explained by higher body weight. Several previous studies have shown an inverse correlation between serum total testosterone and body weight and dyslipidaemia (13, 14), where morbid obesity (BMI > 35 kg/m²) was also accompanied by a decrease in free testosterone (15).

Testosterone levels in the community change with the biorhythm of its secretion, genetic factors and health status, and decrease with increasing age, body weight and smoking, where the latter had decreased, and dietary habits changed as judged from lipid levels (13, 14, 15, 16). The blood sampling was performed using a similar method, at a similar time of the day and with similar routines, speaking against any laboratory or diurnal variation as an explanation of these changes (16).

Conversion of testosterone to oestradiol takes place in the adipose tissue. Body fat was lower but serum oestradiol was unaltered in the men of 2008 compared with the men of similar age in 1995.

Of the factors studied here, the only possible explanation of the lower levels of serum testosterone in men in 2008 could be the concomitantly lower levels of serum lipids. This could be confirmed by the parallel secular trends for lipids and testosterone in the present study. Cholesterol is essential as a substrate for the synthesis of testosterone in the testes and adrenal glands. The generally lower serum triglyceride and cholesterol concentrations may contribute to the decreased testosterone levels, but this was not seen in women. Lipid-lowering agents had increased by up to 15%, but they are not considered to be of major importance for blood lipid levels in the general population. Serum lipids have declined in the general population throughout the last 15 years, irrespective of the use of lipid-lowering agents (17, 18).

Smoking and coffee consumption are not likely to have contributed to these secular trends in sex hormones, as these lifestyle factors have declined (smoking was decreased in the whole 2008 cohort) or remained unchanged in the different age groups.

Physical activity at work or during leisure time can hardly explain the decline in testosterone nowadays as there was no concordance between the direction of physical activity and testosterone levels. It has been put forward that environmental endocrine-disrupting compounds, such as phthalates, a class of plasticisers and solvents commonly found in clothes, could affect testosterone levels negatively (7).

Secular trends were seen in both fracture incidence and fracture type in postmenopausal women but not in men over the last 13 years. A decrease in HRT use and serum oestradiol may be one explanation, especially regarding the increase in vertebral trabecular bone fractures. This hypothesis is supported by the present finding of fewer HRT users among fractured women than non-fractured women in 1995. The indication for HRT in the general female population at that time was mainly postmenopausal symptoms. In 2008, on the contrary, the few cases with HRT were fractured women.

These results indicate that the effect of oestrogen withdrawal seems to be rapid. The present secular trends, as a result of the almost complete cessation of HRT prescription, seem to have led to a dramatic increase in the fracture outcome and change in fracture panorama, i.e. the increase in trabecular bone fractures, which are directly sex hormone dependent. It is, however, unclear to what extent the decreased serum testosterone in the young women of the present study may contribute to the future fracture incidence. A study by Ali *et al.* (19) showed that neither HRT nor age affects testosterone and SHBG in women. The results support the importance of oestrogen in bone synthesis (20).

Concomitantly higher use of tranquilisers in women of 2008 and physical activity during leisure time, indicative of an increased fall risk exposure, may also have contributed to the increased fracture frequency (21, 22, 23, 24). Undoubtedly, leisure time physical activity has beneficial effects on muscle tone and bone mass (9, 25), as was also seen on QUS in the present women above 45 years.

More fractures in women in the age group 35–64 nowadays, while still working, is a worrying matter. Every third to fourth woman had fractured at least once, compared with every fifth woman in the 1990s. Furthermore, physical activity at work was lower in women in 2008, indicating less general daily activity. The results also show that despite greater bone mass and more treatment for osteoporosis in the 45–64-year-old women in 2008, they had sustained more fractures. QUS correlated well with dual-energy X-ray absorptiometry (26). However, bone density is considered a fairly weak predictor of fracture (12, 27).

The 10-year probability of suffering a fracture was higher in postmenopausal women of 2008 than in women of the same category in 1995 probably due to more fractures in women of 2008 as BMI was unaltered and smoking had declined. Previous fracture had the greatest weight in the FRAX model (12).

The higher WHR in postmenopausal women in 2008 could be explained by the increased incidence in vertebral fractures and, thus, by their reduced height as the lowest rib margin comes closer to the iliac crest leading to a higher waist circumference after a vertebral compression.

On the contrary, no change in the fracture incidence was seen in men, despite the lower serum free testosterone levels in 2008. Hip fractures occur after the age of 70 (25). This is beyond the ages studied in the present study, but has been shown in a previous report from the same region (25). Thus, the observed secular trend of decreased testosterone levels in men in 2008 is likely to be mirrored in the coming 10–20 years by an increased fracture incidence if the male sex hormone is of importance to the bone structure. Secular trends in hip fractures are being discussed (28). Hip fractures mainly occur due to falls, and nutritional factors are important for the cortical bone.

Some of the analyses in Tables 1 and 2 show fairly large differences (total testosterone in the youngest men, smoking and IGF1), but due to the small sample size they did not reach statistical significance.

A major limitation of this study was the small sample size, especially in 2008. The optimal study design had been to compare the random population sample from 1995 with a new random sample of similar age in 2008. Although the subjects in the present 2008 re-examination were part of the large 1995 cohort, it was still different subjects that were compared with respect to secular trends, 13 years apart. Bioimpedance is not a fully reliable method, but the relationship in FFM and fat could at least be studied. The RIA method for analysis of sex hormones is not fully reliable either, compared with mass spectrometry, but it was the method of choice in 1995 worldwide. To test our hypothesis of possible secular trends, it was necessary to use the same method in 2008. Vitamin D was not measured in 1995 but the percentage of subjects with 25-OH-vitamin D deficiency (<25 nmol/l) was 3% in 2008. This is not more than in another random population sample, the WHO MONICA cohort in 1985 (29), or than in a Danish study with blood drawn in year 2000, with 13.8% shown to have a deficiency (30).

The strengths of the study are the fairly long time period of 13 years and a random population from young age to upper-middle age that it represents. In addition, all subjects were traced with regard to fracture incidence through hospital registers on both occasions. The pre-, peri- and postmenopausal ages studied are the important ages for changes in sex hormones and the onset of fractures in women. Bone measurement with

QUS was well correlated with dual-energy X-ray absorptiometry (26).

In conclusion, secular (age independent) trends were seen with lower serum testosterone in the 2008 men compared with men of the same age in 1995, independently of body weight. Fracture incidence, physical activity and use of tranquilisers were similar. In the postmenopausal women of 2008, a higher fracture incidence was seen, along with more vertebral compression. Lower HRT use, lower serum oestradiol and higher fall risk exposure due to more tranquiliser use and leisure time physical activity in women may explain the results.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

P Trimpou has performed the examination of all subjects who participated in the re-examination of the WHO MONICA 2008 cohort and has written this paper. G Lindstedt was responsible for the biochemical analyses in 1995 and A Lindahl and G Oleröd for the analyses in 2008. L Wilhelmsen was the principal investigator of the WHO MONICA 1995 cohort and contributed with statistical advice and co-authoring. K Landin-Wilhelmsen was the principal investigator of the re-examination of the WHO MONICA 2008 cohort and contributed with statistical advice, co-authoring and financial support.

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