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Smoking as a determinant for plasma levels of testosterone, androstenedione, and DHEAs in postmenopausal women

Jonas Manjer[1,2], Robert Johansson[3], & Per Lenner[4]
[1]*The Malmö Diet and Cancer Study, Departments of Community Medicine and Medicine;* [2]*Department of Surgery, Malmö University Hospital, Malmö;* [3]*Oncological Centre;* [4]*Department of Radiation Sciences, Oncology, Norrland University Hospital, Umeå, Sweden*

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Abstract. The aim of the present study was to investigate whether current smoking, ex-smoking, or amount of current smoking among postmenopausal women was associated with plasma levels of testosterone, and rostenedione, and DHEAs. About 65,000 women in Sweden have participated in two population-based prospective cohort studies where blood samples were collected at baseline, and information on life-style, reproductive history and anthropometrical measurements were available. The present study was based on 407 control subjects from a previous nested case–control study on the relation between steroid hormone levels and risk of breast cancer. A multivariate logistic regression analysis, adjusted for potential confounders, was used in order to obtain odds ratios (OR) with 95% confidence intervals. There was a high risk of high testosterone levels (above the median) in current vs. never smokers, OR: 1.85 (1.06–3.23). Risk of high testosterone levels increased by amount of daily smoking (increments of 10 cigarettes/day), OR: 1.55 (1.02–2.37). Ex-smoking was associated with high testosterone levels, OR: 1.56 (0.98–2.47). Current smoking and increasing amount of current smoking were weakly associated with high androstenedione levels. However, these associations did not reach statistical significance. No association was seen between smoking habits and DHEAs levels. We conclude that current smoking, and increasing amount of daily smoking, is associated with high testosterone levels.

Key words: Androstenedione, DHEAs, Smoking, Testosterone, Women

Abbreviations: BMI = body mass index; CI = confidence interval; DHEAs = Dehydroepiandrosterone sulphate; DL = detection limit; HRT = hormonal replacement therapy; MDCS = The Malmö Diet and Cancer Study; MSP = The Mammary Screening Project; NSHDS = The Northern Sweden Health and Disease Study; OC = oral contraceptives; OR = odds ratio; VIP = The Västerbotten Intervention Project

Introduction

High androgen levels have been associated with increased risk of breast cancer [1], hyperinsulinaemia [2], and diabetes mellitus [3]. It has also been suggested that androgen levels are related to conditions such as bone density [4] and arteriosclerosis [5]. Several studies have investigated determinants for androgen levels in men, but few large studies have included women. The potential association between smoking and androgen levels in postmenopausal women has been studied in at least 14 previous studies [6–19], but many of these studies were performed in populations where smoking was relatively uncommon, and most studies were small, 8 including less than 50 smoking exposed women (current or ex-smoker, i.e. ever smokers) [6, 8–10, 12, 13, 15, 17]. Almost all previous studies have used only two categories to classify smoking exposure; current smokers and non-smokers, the latter category including both never and ex-smokers [6, 8–15, 17, 19]. Only two studies have reported results related to different amounts of current smoking [7, 12].

About 65,000 women in Sweden have participated in two population-based prospective cohort studies where blood samples were collected at baseline: The Malmö Diet and Cancer Study (MDCS) [20], and The Northern Sweden Health and Disease Study (NSHDS) [21]. Smoking is common in the background populations, about 30% of the population were daily smokers in Malmö, and about 20% in the north of Sweden [22].

The aim of the present study was to investigate whether current smoking, ex-smoking or amount of current smoking among postmenopausal women was associated with plasma levels of testosterone, androstenedione, and DHEAs.

Materials and methods

Study cohorts

The Malmö Diet and Cancer Study (MDCS) and the Northern Sweden Health and Disease Study (NSHDS) are population-based cohorts from Sweden including about 65,000 women [1, 20, 21, 23]. The Northern Sweden Health and Disease Study consists of three sub-cohorts: the Västerbotten Intervention Project (VIP), the Umeå component of the MONICA study, and the Mammary Screening Project (MSP). Both the MDCS and the NSHDS collected blood samples at baseline, and assessed information on anthropometry, life-style factors, and reproductive history. Smoking habits were not assessed in the MSP [1, 20, 21, 23].

Selection of subjects

This study included control subjects from a previous nested case–control study on the relation between steroid hormone levels and risk of breast cancer [1]. Ethical clearance was given by ethical committees at Lund University and Umeå University. Menopausal definition, case retrieval, and matching of controls have been previously described [1]. Altogether, 344 cases and 676 controls, postmenopausal at baseline, were identified.

Control subjects can be regarded as a randomised sample from the postmenopausal population, as there were breast cancer cases of all ages in the postmenopausal period, and all breast-cancer free individuals were eligible to be selected as a control. Among 676 control subjects, information on smoking had been assessed in the 514 controls from the MDCS, the VIP and the MONICA cohorts. Menopausal status was confirmed using FSH and estradiol levels, as previously described, leaving 482 control subjects with postmenopausal levels [1]. In order to get reliable information on current use of hormonal replacement therapy (HRT) at baseline, women with missing information on this issue, and those affirming ever use of HRT in the NSHDS (current use was not assessed in this cohort), were excluded, leaving 408 women. One subject had no information on smoking habits. Hence, a total of 407 control subjects were available for the present study.

Laboratory analyses

Following venepuncture, blood was separated into different components. Plasma samples were stored in the biological bank at −80 °C [24]. Following extraction with ether, radio-immunoassay (using ^{125}I tracers) was used in order to analyse testosterone (detection limit (DL): 0.10 nmol/l), androstenedione (DL: 0.14 nmol/l), and DHEAs (DL: 0.20 μmol/l) (Department of Clinical Chemistry, Malmö University Hospital, Malmö, Sweden) [24]. Reference values among postmenopausal women which were used in clinical practice were, for testosterone: 0.3–2.5 nmol/l, for androstenedione: 1.0–8.0 nmol/l, and for DHEAs: 1.0–12.0 nmol/l.

Laboratory procedures have been previously described in detail [1]. Inter-assay coefficients of variation were below 15% for all three hormones at high and low levels, respectively. Corresponding intra-assay coefficients for testosterone and androstenedione were below 10%, and below 16% for DHEAs. The amount of biological material was limited in some subjects. Eventually, testosterone levels could be determined in 387 women, and androstenedione and DHEAs levels in 389 subjects.

Statistical methods

Levels of testosterone, androstenedione, and DHEAs were dichotomised in order to define subjects with high vs. low levels of these hormones. Cut-off points (medians) were defined separately for the MDCS and the NSHDS-cohorts, Table 2. A number of potential confounders were investigated in relation to low vs. high levels of the studied hormones. These factors included standard demographic parameters such as age at baseline and educational level, and information relevant to the laboratory analyses, i.e. storage time and sub-cohort. Sowers et al. [18] have reported that testosterone levels are associated with BMI, previous bilateral oophorectomy, use of oral contraceptives (OC) and use of HRT. In addition, a weak, not statistically significant, relation was seen between alcohol consumption and testosterone levels [18]. A large part of all testosterone, and a minor part of androstenedione and DHEAs, in women is produced by the ovary [7, 16, 18]. This study included information related to previous and present ovary function, i.e. age at menarche, parity, age at first childbirth and age at menopause.

Median and range (5th to 95th percentiles) were given separately for the two centres. Levels of the studied hormones in current, ex, and ever smokers were compared to those in never smokers using the Mann-Whitney test. This, and all other tests, was two-tailed and p-values less than 0.05 were considered statistically significant.

The odds of high vs. low hormonal levels in current, ex- and ever smokers was compared to corresponding odds in never smokers yielding odds ratios (ORs) with a confidence interval (CI) of 95%. Unconditional logistic analysis was used to obtain OR adjusted for age at baseline. A second model included all potential confounders listed above. As some strata had no or very few subjects who had undergone bilateral oophorectomy, this factor was not included in the multivariate analysis. Risk of high hormonal levels in relation to amount of current smoking was analysed accordingly. Amount of cur-

Table 1. Characteristics of women with low and high levels of androgens

Factor	Testosterone levels		Androstenedione levels		DHEAs levels	
	Low (n = 194)	High (n = 193)	Low (n = 194)	High (n = 195)	Low (n = 194)	High (n = 195)
	Column % *(mean and SD in italics)*					
Age at baseline (years)	*59.9 (5.3)*	*60.4 (5.6)*	*60.9 (5.2)*	*59.4 (5.5)*	*61.2 (5.3)*	*59.1 (5.4)*
Storage time (years)	*6.4 (1.5)*	*6.3 (1.6)*	*6.4 (1.6)*	*6.3 (1.6)*	*6.4 (1.6)*	*6.3 (1.6)*
Sub-cohort						
MDCS	86.1	86.5	86.6	86.2	86.6	86.2
NSHDS-VIP	12.9	11.9	11.9	12.8	12.9	11.8
NSHDS-MONICA	1.0	1.6	1.5	1.0	0.5	2.1
Education						
O-level college	80.9	74.9	76.8	78.8	79.9	75.6
A-level college	7.2	7.3	7.7	6.7	7.2	7.3
University	11.9	17.8	15.5	14.5	12.9	17.1
Age at menarche (years)	*13.5 (1.6)*	*13.5 (1.5)*	*13.5 (1.6)*	*13.5 (1.5)*	*13.5 (1.5)*	*13.4 (1.5)*
Parity (number of children)						
0	13.2	7.3	12.0	8.3	13.2	7.3
1	20.0	24.1	22.0	21.9	22.1	21.8
2	43.7	45.0	43.5	45.8	42.6	46.6
3	15.8	17.3	17.3	15.6	15.3	17.6
>4	7.4	6.3	5.2	8.3	6.8	6.7
Age at first childbirth (years)						
Nullipara	13.2	7.3	12.0	8.3	13.2	7.3
⩽20	22.6	16.8	19.4	19.8	24.7	14.5
>20 –⩽25	33.7	32.5	30.4	36.5	28.4	38.3
>25 –⩽30	23.2	34.0	28.8	28.1	25.8	31.1
>30	7.4	9.4	9.4	7.3	7.9	8.8
Bilateral oophorectomy	2.6	0.0	2.1	0.5	2.1	0.5
Age at menopause (years)	*48.9 (5.3)*	*49.0 (4.6)*	*49.3 (5.0)*	*48.6 (4.9)*	*49.2 (5.1)*	*48.7 (4.8)*
Body mass index (kg/m^2)	*25.3 (3.8)*	*26.3 (4.9)*	*25.6 (4.1)*	*26.0 (4.7)*	*25.8 (4.4)*	*25.8 (4.4)*
Ever use of OC	38.1	31.3	34.0	35.6	30.1	39.5
Current use of HRT	16.5	13.5	16.0	14.4	14.4	15.9
Alcohol consumption						
Teetotaler	17.0	17.6	17.5	16.9	19.6	14.9
Something last year	17.0	15.5	19.1	13.8	21.1	11.8
Something last month	66.0	66.8	63.4	69.2	59.3	73.3

MDCS, The Malmö Diet and Cancer Study; NSHDS, The Northern Sweden Health and Disease Study; VIP, Västerbotten intervention project; MONICA, The Umeå component of the MONICA study.

rent smoking was expressed as both categorical and continuous variables.

The relation between smoking habits and androgen levels were further investigated using multiple linear regression analysis. Androgen levels were approximately normally distributed. Continuous and dichotomous variables were entered as in the analyses above. Variables for sub-cohort, education, age at first childbirth, and alcohol consumption in addition to categorical outcome variables were transformed and entered as multiple categorical variables. Partial regression coefficients (β_i) were reported together with a 95% confidence interval and corresponding *p*-values.

Results

The distribution of known and potential risk factor for breast cancer among women with low and high levels of androgens respectively is given in Table 1. Mean and range of the studied hormones is reported in Table 2.

The multivariate analysis showed a statistically significant risk of high testosterone levels in current vs. never smokers, OR: 1.85 (1.06–3.23), Table 3. Excluding HRT users (n = 60) from the analysis, the result was similar, OR: 1.85 (1.00–3.44). Risk of high testosterone levels increased by amount of daily smoking (increments of 10 cigarettes/day), OR: 1.55 (1.02–2.37), Table 4. Excluding HRT users, the risk was marginally lower, OR: 1.44 (0.91–2.28). Ex-smoking was associated with high testosterone levels, but this association did not reach statistical significance, Table 3. These findings were confirmed by the multiple linear analyses, Tables 3 and 4.

Androstenedione levels were higher in current smokers as compared to never smokers (Table 2), and the multiple linear regression analysis yielded a statistically significant association, (Table 3). However, this association did not reach statistical significance when outcome was defined as high androstenedione level, Table 3, and there was no statistically significant dose–response relation between amount of current smoking and androstenedione levels, Table 4.

Discussion

Current smoking and increasing number of cigarettes smoked per day were associated with high testosterone levels. The results also indicate that there may be an association between ex-smoking and high testosterone levels, and between current smoking and high androstenedione levels.

Several methodological issues have to be considered. One concerns the validity of a single value as a measurement of mean hormonal levels. Short-time variation seems to be small regarding sex-steroid hormones in postmenopausal women [26], and several studies have found that a single measurement of sex steroid hormones reflects levels over long time [27, 28]. The laboratory has a long experience with

Table 2. Means and values for the 5th and 95th percentiles, of androgens in relation to smoking habits

		MDCS			NSHDS		
Hormone	Smoking status	No.	Median (5–95 percentiles)	Compared to never smokers (*p*-value)	No.	Median (5–95 percentiles)	Compared to never smokers (*p*-value)
Testosterone (nmol/l)							
	Never	162	1.10 (0.58–1.89)	ref.	27	1.44 (0.74–3.00)	ref.
	Current	91	1.21 (0.49–2.21)	0.17	16	1.41 (0.90–2.58*)	0.87
	Ex	81	1.15 (0.67–1.83)	0.93	10	1.56 (1.14–2.25*)	0.75
	Ever (current + ex)	172	1.16 (0.59–1.89)	0.42	26	1.47 (0.92–2.55)	0.78
	All	334	1.13 (0.59–1.89)	–	53	1.44 (0.88–2.71)	–
Androstenedione (nmol/l)							
	Never	163	2.08 (1.05–3.58)	ref.	27	3.14 (0.99–9.41)	ref.
	Current	91	2.48 (1.05–5.17)	0.01	16	3.53 (1.34–7.31*)	0.56
	Ex	82	2.01 (1.10–3.76)	0.27	10	3.45 (2.36–4.98*)	0.30
	Ever (current + ex)	173	2.13 (1.08–4.51)	0.33	26	3.45 (1.39–7.25)	0.34
	All	336	2.12 (1.09–4.21)	–	53	3.21 (1.24–7.18)	–
DHEAs (μmol/l)							
	Never	163	2.59 (0.69–7.06)	ref.	27	2.75 (0.30–8.99)	ref.
	Current	91	2.85 (0.49–6.70)	0.51	16	2.96 (0.41–8.60*)	0.86
	Ex	82	2.07 (0.39–5.90)	0.22	10	3.88 (1.77–5.92*)	0.14
	Ever (current + ex)	173	2.40 (0.47–6.52)	0.76	26	3.30 (0.45–8.56)	0.37
	All	336	2.49 (0.51–6.49)	–	53	2.84 (0.49–8.52)	–

Presented separately for The Malmö Diet and Cancer Study (MDCS) and The Northern Sweden Health and Disease Study (NSHDS). *Maximum value.

Table 3. Crude and adjusted odds ratios (ORs), with confidence intervals (CIs) of 95%, for high vs. low androgen levels in different smoking categories

						Multiple regression analysis*	
Hormone	Smoking status	Low levels N	High levels N	Crude OR	Adjusted OR*	partial regression coefficient, β_i (95% CI)	*p*-value
Testosterone							
	Never	103	86	1.00	1.00	Baseline	–
	Current	48	59	1.47 (0.91–2.37)	1.85 (1.06–3.23)	0.13 (0.02–0.23)	0.02
	Ex	43	48	1.34 (0.81–2.21)	1.32 (0.75–2.30)	0.004 (−0.10–0.11)	0.94
	ever (current + ex)	91	107	1.41 (0.94–2.10)	1.56 (0.98–2.47)	0.07 (−0.02–0.16)	0.15
Androstenedione							
	Never	98	92	1.00	1.00	Baseline	–
	Current	41	66	1.71 (1.06–2.78)	1.42 (0.82–2.47)	0.33 (0.03–0.64)	0.03
	Ex	55	37	0.72 (0.43–1.19)	0.65 (0.37–1.14)	−0.09 (−0.39–0.22)	0.58
	ever (current + ex)	96	103	1.14 (0.77–1.70)	0.97 (0.61–1.53)	0.13 (−0.13–0.40)	0.33
DHEAs							
	Never	94	96	1.00	1.00	Baseline	–
	Current	50	57	1.12 (0.69–1.79)	0.84 (0.48–1.49)	−0.18 (−0.68–0.32)	0.47
	Ex	50	42	0.82 (0.50–1.35)	0.65 (0.37–1.16)	−0.30 (−0.80–0.21)	0.25
	ever (current + ex)	100	99	0.97 (0.65–1.44)	0.74 (0.46–1.19)	−0.24 (−0.65–0.18)	0.26

Multiple linear regression analysis on the relation between smoking habits and androgen levels.
*Adjusted for age at baseline, storage time, sub-cohort, educational level, age at menarche, parity, age at first childbirth, age at menopause, BMI, use of OC, use of HRT and alcohol consumption.

regard to analysis of the studied hormones, and we consider the coefficients of variation to be acceptable.

Information on smoking habits has a high reliability according to a previous report from the MDCS [23]. Validity of information on smoking was reported to be good in an earlier study from Malmö where questionnaire information was compared to plasma levels of carboxy-haemoglobin [29].

Subjects were selected from control subjects in a previous case–control study nested within a population-based prospective cohort. This ought to minimize potential selection and detection bias. One limitation of this study is, however, that only postmenopausal women were studied. Whether the observed association apply to premenopausal women cannot be assessed by this material.

Potential confounders include age at baseline, storage time, sub-cohort, educational level, BMI, alcohol consumption and reproductive history. All these factors were included in the multivariate analysis, and ought not to have affected the observed associations. When restricted to women with no HRT at baseline, results were similar, although confidence intervals were broader, probably due to decreased statistical power.

Current smoking was associated with high testosterone levels in this study. This relationship has been investigated in at least eight previous studies among postmenopausal women [6–10, 16–18]. Out of them, 3 found a statistically significant positive association [6, 16, 18], among them was the largest previous study including 194 smoking exposed women [18]. One study indicated a positive association [8], but this, and 3 out of 4 studies that showed no association at all, included less than 50 smokers. Hence, lack of statistical power may have been a problem. A meta-analysis based on 6 of these studies [6–10, 17], including 150 smokers, showed slightly higher testosterone levels in smokers, but this association did not reach statistical significance. We found a dose–response relationship between the amount of current smoking and testosterone levels. One previous study has investigated this relationship, but it did not find such a trend [7]. Previous studies often compare current smoking with non-smoking, including ex-smokers in the reference category [6, 8, 9, 17]. If ex-smoking is positively associated with high androgen levels, as our study suggests, this would obscure a true risk associated with current smoking.

There was a slight increase in risk of high androstenedione levels in current smokers, an association that was stronger in heavy smokers. Smoking and androstenedione levels have been assessed in at least 11 studies [6–11, 13–17]. All of them found a positive association, statistically significant in five [6, 7, 11, 15, 16]. Three of these 5 were also the largest studies, with at least 50 smokers in each. A meta-analysis based on 10 of these studies [6–11, 13–15, 17] found that smokers had statistically significantly higher androstenedione levels as compared to non-smokers. Our study indicated a dose–response relation between amount of daily smoking and androstenedione levels, in line with the only previous study investigating this association [7].

Table 4. Crude and adjusted odds ratios (ORs), with confidence intervals (CIs) of 95%, for high vs. low androgen levels in different categories of daily smoking

						Multiple regression analysis*	
Hormone	Smoking status (number of cigarettes/day)	Low levels N	High levels N	Crude OR	Adjusted OR*	partial regression coefficient, β_i (95% CI)	*p*-value
Testosterone							
	Never (0/day)	103	86	1.00	1.00	Baseline	–
	⩽14/day	26	28	1.29 (0.70–2.36)	1.63 (0.82–3.24)	−0.05 (−0.13–0.03)	0.25
	⩾15/day	22	25	1.36 (0.72–2.58)	2.05 (0.95–4.44)	0.05 (−0.03–0.14)	0.21
	Continuous variable (per 10/day)	–	–	1.26 (0.89–1.77)	1.55 (1.02–2.37)	0.11 (0.03–0.20)	0.01
Androstenedione							
	Never (0/day)	98	92	1.00	1.00	Baseline	–
	⩽14/day	23	31	1.44 (0.78–2.64)	1.27 (0.64–2.53)	−0.07 (−0.31–0.16)	0.53
	⩾15/day	17	30	1.88 (0.97–3.64)	1.57 (0.71–3.47)	0.10 (−0.13–0.33)	0.39
	Continuous variable (per 10/day)	–	–	1.41 (0.99–2.00)	1.23 (0.80–1.88)	0.18 (−0.05–0.42)	0.13
DHEAs							
	Never (0/day)	94	96	1.00	1.00	Baseline	–
	⩽14/day	26	28	1.05 (0.58–1.93)	0.87 (0.43–1.80)	−0.11 (−0.49–0.27)	0.56
	⩾15/day	23	24	1.02 (0.54–1.93)	0.63 (0.29–1.40)	0.14 (−0.24–0.52)	0.48
	Continuous variable (per 10 / day)	–	–	1.10 (0.78–1.54)	0.86 (0.56–1.32)	−0.09 (−0.47–0.29)	0.64

Multiple linear regression analysis on the relation between smoking habits and androgen levels.
*Adjusted for age at baseline, storage time, sub-cohort, educational level, age at menarche, parity, age at first childbirth, age at menopause, BMI, use of OC, use of HRT and alcohol consumption.

No association was seen in this study between smoking and DHEAs levels. This relationship has been investigated in at least seven studies [6, 7, 12, 13, 15–17]. Contrary to our study, most others found a positive association between smoking and high DHEAs levels [6, 7, 13, 15–17]. A positive association between smoking and DHEA levels has also been suggested by Trichopoulou et al. [19]. The relation was statistically significant in three of these studies [7, 15, 16] and also in one meta-analysis including a total of six of these studies [6, 7, 12, 13, 15, 17]. A dose response relation was observed in the only previous study investigating this relation [7].

Sowers et al. [18] have hypothesised that increased levels of testosterone in smokers may be an effect of decreased steroid hormone metabolism. One possible mechanism is that testosterone and androstenedione are degraded by enzymes that may be inhibited by carbon monoxide present in tobacco smoke [8]. An alternative explanation is that nicotine from tobacco smoke induces ACTH production, which in turn leads to higher levels of adrenal androgens (androstenedione and DHEAs) [7]. As androstenedione and DHEAs are precursors in steroid hormone synthesis, it is possible that this may also affect plasma levels of testosterone [12].

This study indicates that androgens may be part of, or may modify, the association between smoking and conditions such as breast cancer, osteoporosis, arteriosclerosis, and metabolic disturbances such as hyperinsulinaemia.

We conclude that current smoking, and increasing amount of daily smoking, is associated with high testosterone levels.

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References

1. Manjer J, Johansson R, Berglund G, et al. Postmenopausal breast cancer risk in relation to sex steroid hormones, prolactin and SHBG. Cancer Causes Control 2003; 14: 599–607.
2. Maturana MA, Spritzer PM. Association between hyperinsulinemia and endogenous androgen levels in Pre- and postmenopausal women. Metabolism 2002; 51: 238–243.
3. Andersson B, Marin P, Lissner L, Vermeulen A, Bjorntorp P. Testosterone concentrations in women and men with NIDDM. Diabetes Care 1994; 17: 405–411.
4. Zofkova I, Bahbouh R, Hill M. The pathophysiological implications of circulating androgens on bone mineral density in a normal female population. Steroids 2000; 65: 857–861.

5. Golden SH, Maguire A, Ding J, et al. Endogenous postmenopausal hormones and carotid atherosclerosis: a case–control study of the atherosclerosis risk in communities cohort. Am J Epidemiol 2002; 155: 437–445.
6. Friedman AJ, Ravnikar VA, Barbieri RL. Serum steroid hormone profiles in postmenopausal smokers and nonsmokers. Fertil Steril 1987; 47: 398–401.
7. Khaw KT, Tazuke S, Barrett-Connor E. Cigarette smoking and levels of adrenal androgens in postmenopausal women. N Engl J Med 1988; 318: 1705–1709.
8. Longcope C, Johnston Jr, CC. Androgen and estrogen dynamics in pre- and postmenopausal women: a comparison between smokers and nonsmokers. J Clin Endocrinol Metab 1988; 67: 379–383.
9. Cauley JA, Gutai JP, Kuller LH, LeDonne D, Powell JG. The epidemiology of serum sex hormones in postmenopausal women. Am J Epidemiol 1989; 129: 1120–1131.
10. Slemenda CW, Hui SL, Longcope C, Johnston Jr, CC. Cigarette smoking, obesity, and bone mass. J Bone Miner Res 1989; 4: 737–741.
11. Schlemmer A, Jensen J, Riis BJ, Christiansen C. Smoking induces increased androgen levels in early post-menopausal women. Maturitas 1990; 12: 99–104.
12. Key TJ, Pike MC, Baron JA, et al. Cigarette smoking and steroid hormones in women. J Steroid Biochem Mol Biol 1991; 39: 529–534.
13. Cassidenti DL, Pike MC, Vijod AG, Stanczyk FZ, Lobo RA. A reevaluation of estrogen status in postmenopausal women who smoke. Am J Obstet Gynecol 1992; 166: 1444–1448.
14. Austin H, Drews C, Partridge EE. A case–control study of endometrial cancer in relation to cigarette smoking, serum estrogen levels, and alcohol use. Am J Obstet Gynecol 1993; 169: 1086–1091.
15. Baron JA, Comi RJ, Cryns V, Brinck-Johnsen T, Mercer NG. The effect of cigarette smoking on adrenal cortical hormones. J Pharmacol Exp Ther 1995; 272: 151–155.
16. Bancroft J, Cawood EH. Androgens and the menopause; a study of 40–60-year-old women. Clin Endocrinol (Oxf) 1996; 45: 577–587.
17. Law MR, Cheng R, Hackshaw AK, Allaway S, Hale AK. Cigarette smoking, sex hormones and bone density in women. Eur J Epidemiol 1997; 13: 553–558.
18. Sowers MF, Beebe JL, McConnell D, Randolph J, Jannausch M. Testosterone concentrations in women aged 25–50 years: associations with lifestyle, body composition, and ovarian status. Am J Epidemiol 2001; 153: 256–264.
19. Trichopoulou A, Bamia C, Kalapothaki V, Spanos E, Naska A, Trichopoulos D. Dehydroepiandrosterone relations to dietary and lifestyle variables in a general population sample. Ann Nutr Metab 2003; 47: 158–164.
20. Manjer J, Carlsson S, Elmstahl S, et al. The Malmö Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. Eur J Cancer Prev 2001; 10: 489–499.
21. Chajes V, Hulten K, Van Kappel AL, et al. Fatty-acid composition in serum phospholipids and risk of breast cancer: An incident case–control study in Sweden. Int J Cancer 1999; 83: 585–590.
22. The National Board of Health and Welfare. [Public Health Report 1997] (in Swedish). Stockholm, Sweden: The National Board of Health and Welfare, 1998.
23. Manjer J, Elmståhl S, Janzon L, Berglund G. Invitation to a population-based cohort study: differences between subjects recruited using various strategies. Scand J Public Health 2002; 30: 103–112.
24. Pero RW, Olsson A, Berglund G, Janzon L, Larsson SA, Elmståhl S. The Malmö biological bank. J Intern Med 1993; 233: 63-67.
25. Thorell J, Larson SM. Radioimmunoassay and Related Techniques. Methodology and Clinical Applications. St Louis, USA: CV Mosby Company, 1978.
26. Lonning PE, Dowsett M, Jacobs S, Schem B, Hardy J, Powles TJ. Lack of diurnal variation in plasma levels of androstenedione, testosterone, estrone and estradiol in postmenopausal women. J Steroid Biochem 1989; 34: 551–553.
27. Micheli A, Muti P, Pisani P, et al. Repeated serum and urinary androgen measurements in premenopausal and postmenopausal women. J Clin Epidemiol 1991; 44: 1055–1061.
28. Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2–3-year period. Cancer Epidemiol Biomarkers Prev 1995; 4: 649–654.
29. Janzon L, Lindell S-E, Trell E, Larme P. Smoking habits and carboxyhaemoglobin: A cross-sectional study of an urban population of middle-aged men. J Epidemiol Community Health 1981; 35: 271–273.

Address for correspondence: Jonas Manjer, Department of Community Medicine, Unit of Epidemiology, Malmö University Hospital, Lund University, SE-205 02 Malmö, Sweden
Phone: +46-(0)-40-333435
Fax: +46-(0)-40-336215
E-mail: jonas.manjer@smi.mas.lu.se