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Reproductive Parameters of Community-Dwelling Men From 2 Regions in Flanders Are Associated With the Consumption of Self-Grown Vegetables

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ABSTRACT: Although regional differences in semen parameters have been described, little is known about the etiologic factors underlying these variations in male fertility status. We previously reported people from a rural area (Peer) in Flanders to have lower sperm parameters and free testosterone than men from the city of Antwerp. In the present study, our objectives were to investigate to what extent these differences were associated with lifestyle or environmental factors. People in Peer were slightly older and had a higher body mass index, factors known to affect testosterone concentrations but not sperm parameters. People consuming locally produced vegetables (n = 37 of 94) but not fruit had significantly lower serum free testosterone and luteinizing hormone (LH) (both $P = .04$) and nonsignificantly lower follicle-stimulating hormone (FSH) ($P = .05$). Per unit increase of monthly intake of locally produced vegetables, free testosterone declined by 0.7% ($P = .01$) and sperm concentration by 2.3% ($P = .04$) over the whole range of the explanatory variable, whereas LH declined by 3.6% ($P = .02$), FSH declined by 3.5% ($P = .08$), and sperm morphology by 7% ($P = .002$) in the range of 0–10 consumptions per month. No relationship was found with lifelong exposure to cadmium. These results support a hypothesis of impaired gonadotropic signaling causing the regional difference in reproductive parameters. The surprising strong impact of self-grown vegetable consumption did not seem to be related to soil contamination by cadmium. We could not exclude pesticide exposure by inappropriate application or other factors such as nutritional deficiency, physical activity, or stress as contributors to the observed regional differences.

Key words: Male reproduction, semen quality, testosterone, vegetable consumption, cadmium, sex hormones, pesticides.

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Male fertility depends on both quantitative as well as qualitative aspects of sperm production. The probability of conception increases with increasing sperm concentration and, independently, the proportion of morphologically normal spermatozoa (Bonde et al, 1998). Male-factor infertility manifests itself at adult age, but the etiologic factors may have their origin much earlier in life. Hormonal imbalance during fetal life may lead to inappropriate male gonadal development resulting in the testicular dysgenesis syndrome (TDS), the hypothesized common underlying entity of which the increased incidence in testicular cancer and possibly hypospadias, cryptorchidism, and decreased sperm quality are thought to be manifestations (Skakkebaek et al, 2001; Sharpe et al, 2003).

Following the highly debated article by Carlsen et al (1992) stating that sperm concentration worldwide has decreased by more than 40% between 1938 and 1991, a number of subsequent reports have refined this conclusion to the existence of regional differences in semen parameters and/or in their decline (Auger and Jouannet, 1997; Swan et al, 2000; Jørgensen et al, 2001, 2002). However, since then, only limited information has emerged on the possible etiologic factors for this observed deterioration of semen quality. In vitro and in vivo experiments have recently shown that a possibly large number of chemicals that have entered the environment in the past era of explosive growth of the chemical industry and agroindustry show hormonal (estrogenic or antiandrogenic) activity and that during fetal exposure some of those are capable of eliciting a spectrum of responses similar to TDS (Wolf et al, 1999; Fisher et al, 2003). Nevertheless, modern adult lifestyle-associated factors such as stress, sedentary

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work, smoking, alcohol consumption, or diet may equally affect male fertility (Bonde and Storgaard, 2002). However, the complex interplay between these factors, the environment, and genetic predisposition during fetal life as well as at adulthood hampers the assessment of their respective roles in male reproductive failure (Sharpe and Franks, 2002).

In 1999, a project consortium investigated the applicability of biomarkers of exposure and of effect in 3 cohorts of different age categories from 2 regions in Flanders (a Flemish Environment and Health [FLEHS] study) with a suspected difference in environmental exposure. We observed a striking lower semen quality in men aged 20–40 years from a rural region compared with those from an urban region that was accompanied by differences in hormone status (Dhooge et al, 2007). We concluded that these observations were unlikely caused by differences in population characteristics such as age and body mass index (BMI). Therefore, in view of the alleged role of environmental and/or lifestyle factors on male fertility, the objective of this study was to further investigate the available data on the associations between such factors and the reproductive parameters in these men.

Methods

Geographic Areas

The selection of 2 areas with a divergent pattern of exposure was based on the presence of potential sources of external contamination and the environmental data available in the different environmental institutions in Flanders. Hoboken and Wilrijk, suburbs of the city of Antwerp (more than 2500 inhabitants per square kilometer), are located 11–13 km southeast of the chemical and petrochemical industry in the seaport of Antwerp. They are the seat of a large primary nonferrous smelter (Hoboken), 2 waste incinerators (Wilrijk), a crematory (Wilrijk), printing works (Wilrijk), and several small or medium-sized enterprises manufacturing electronic equipment, plastics, and nonferrous products. The suburbs are crossed by motorways with a traffic density in excess of 80 000 vehicles per day. The 2 waste incinerators started operating in 1971 and 1980 and had an annual turnover of 23 000 and 110 000 tons, respectively. Their activity was stopped because the emissions of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) exceeded recommendations (0.1 ng/m^3) by 20-fold, and topsoil samples in the area around the incinerators had levels of these compounds up to 35.9 ng international toxic equivalents (I-TEQ) per kilogram of dry weight (De Fré et al, 1999). Hoboken is an area with a history of heavy metal contamination (Nouwen et al, 2004). Peer (fewer than 200 inhabitants per square kilometer), on the other hand, is situated in a rural agricultural area 70 km east of Antwerp, and possible polluting industries are at a minimum distance of about 15 km. Typical industry-related environmental pollution parameters were lower in Peer than in many other places in Flanders (van Larebeke et al, 2004). Pesticide data from Peer are not available. However, according to the Flemish Environment Agency, limited monitoring data for small rivers in the near surroundings show levels exceeding the standards for hexachlorocyclohexane and a yearly recurring pattern of triazine contamination consistent with agricultural activities (Ilse Theuns, e-mail communication, 9 December 2004). Eels from the same rivers had levels of lindane, hexachlorobenzene, and dichloro-diphenyl-trichloroethane (DDT) in their muscle tissue above the 75th percentile of those measured in an extensive study covering 260 locations in Flanders (calculated from Goemans et al, 2003).

Participants

The recruitment and sampling procedure for the 2 populations has been described in detail elsewhere (Dhooge et al, 2007). In short, 2487 short questionnaires on diet, lifestyle, and profession with an accompanying letter were mailed to possible male candidates selected randomly from the municipal population registries of Antwerp and Peer. Of the 744 responders, 203 men were eligible after consideration of the following exclusion criteria: vasectomy, working in a region with environmental characteristics clearly different from the area of residence, commuting over long distances, and having a job with specific risks of exposure (eg, chemical industry, dry cleaning, production, or military airport of Kleine Brogel). The candidates were contacted by telephone to further clarify the study protocol and to recheck their eligibility and willingness to participate. A similar proportion of candidates in Antwerp (82%) and Peer (92%) was contacted before the number of 50 participants within each area was reached; preference was given to nonsmokers and lifelong residents of the area. The overall participation rate was 55%. Two currently smoking men were allowed to enter the study, 1 from each region. Due to a misclassification, an extra eligible person in Peer was included in the study (n = 51). In both areas, no differences in age, having children, eating self-grown vegetables, or being a vegetarian were noted between those that were contacted and those that were not and between those that entered the study and those that refused during the telephone conversation. All partici-

pants gave their informed consent. The ethics committee of the University of Leuven approved the study.

Methods

Semen samples collected by masturbation were processed within 45 minutes at the site of investigation; a seminal smear was prepared by trained nurses, 2 mL of Hayem anticoagulant and preservative solution was added, and the specimen was stored at 4°C. All samples reached the andrology department within 4 days of semen collection. Ejaculate volumes were estimated using a graduated pipette. A single technician scored sperm concentration in duplicate using disposable counting chambers (Cellvision, Heerhugowaard, The Netherlands) or sperm morphology on air-dried Papanicolaou-stained seminal smears. Using the morphology method recommended by the 1992 World Health Organization manual, only sperm with absolutely no defects were classified as normal. The total sperm count (TSC) was derived by multiplying the individual's sperm concentration and volume.The participants completed a questionnaire to assess lifestyle, social class, use of tobacco and alcohol, and food intake and intake of medicines, similar to the one applied in the adolescent study (Staessen et al, 2001). Nonsmoking participants were classified as ex-smokers if they had smoked at least 1 cigarette a day for at least 1 year; otherwise, they were classified as never-smokers. The amount of animal fat intake per person was calculated from their intake of meat, fish, and dairy products in the year before study by use of Dutch food composition tables (Van Erp-Baart, 1993). Body length and weight were measured at the day of investigation by the nurses.

Venous blood was obtained and centrifuged at the site. Serum was divided into aliquots for determination of markers of hormone status and was stored at −20°C until analysis. Commercial immunoassays were used to determine serum levels of total testosterone (T) (Medgenix, Fleurus, Belgium), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Roche Diagnostics, Vilvoorde, Belgium), sex hormone binding globulin (SHBG; Orion Diagnostica, Espoo, Finland), total 17β-estradiol (E2) (Clinical Assay; DiaSorin, Saluggia, Italy; adapted protocol with use of double amount of serum), and inhibin B (Serotec, Oxford, United Kingdom). The free fractions of T (free T, or fT) and E2 (free E2, or fE2) were calculated from serum total T and SHBG, assuming a fixed albumin concentration using a validated equation (Vermeulen et al, 1999; Szulc et al, 2004). The intra-assay and interassay coefficients of variation for all assays were less than 12%. For every individual the ratio of total T on LH (T/LH) was calculated as nanomoles per international unit (nmol/IU), the ratio of T on E2 (T/E2) as picomoles per picomole (pmol/pmol), and the inhibin B on FSH ratio (inh/FSH) as nanograms per international unit (ng/IU).

Approximately 30 mL of urine was collected for the determination of cadmium concentrations, as described previously (Claeys et al, 1992; Van Hummelen et al, 1993). Urinary measurements were standardized to 1 mmol of creatinine.

Statistical Analyses and Exclusions

Hormone levels are reported as molar units or international units (IU) expressed per liter of serum except for inhibin B, which is expressed as nanograms per liter (ng/L). One participant from Peer had a hormone and sperm profile suggestive of Klinefelter syndrome and was excluded from the dataset, reducing the number in Peer to 50 participants. Gaussian distributed data were described as average and standard deviation (SD). Data that were not normally distributed were described by median and interquartile range (IQR) or log transformed and described by geometric mean (GM) and 95% confidence intervals (95%CI). First, we compared unadjusted means and proportions across the 2 areas by an independent samples *t* test or Mann-Whitney *U* test, and Fisher exact test, respectively. Next, applying analysis of covariance, potentially important covariates were forced into the models irrespective of statistical significance. Finally, we investigated relationships between biomarkers of exposure and biomarkers of effect using scatter plots, Loess regression, and Spearman rank correlation *(r)*. Loess regression has the advantage that it does not require the specification of a function to fit a model to all of the data in the sample and thus can be used to visualize complex relationships between parameters. Dose-effect relations in individuals were subsequently calculated by use of multiple-linear regression. Because of the nonlinear relationship between sperm parameters and the abstinence period, the latter was coded as less than 2 days, between 2 and 4 days, between 4 and 6 days, or higher and corrected for in multiple linear regression. Age was included as a confounder in sperm parameter analyses (Kidd et al, 2001). Statistical analyses were done with SPSS software (version 12.0; SPSS Inc, Chicago, Ill).

Results

The characteristics of the population are presented in Table 1. Participants from Peer were older and had a higher average BMI, resulting from their shorter stature. On an average, in Peer, blood was collected 2 hours later

Table 1. *Characteristics of the population**

	Peer (n = 50)	Antwerp (n = 50)	*P*†
Age, y	34.2 (4.8)	30.9 (6.3)	.004
Anthropometrics			
Body weight, kg	80.5 (12.7)	79.7 (11.9)	.77
Body height, cm	175.2 (4.7)	180.8 (7.2)	<.001
BMI, kg/m^2	26.2 (3.8)	24.3 (3.1)	.009
Self-reported information			
Median residence period, y (IQR)	10.3 (5.2–25.8)	13.5 (6.8–24.8)	.56
Education			
Workers, n (%)	20 (44)	15 (32)	...
Middle class, n (%)	22 (48)	25 (53)	...
Educated professionals, n (%)	4 (9)	7 (15)	.43
Alcohol consumers, n (%)	29 (59)	27 (54)	.69
Median alcohol consumption, g/d (IQR)‡	20.0 (10.0–25.9)	18.2 (9.3–23.3)	.65
Ex-smoker, n (%)	17 (34)	14 (28)	.67
Median years since stopped smoking (IQR)	6.3 (4.0–14.1)	3.9 (2.0–10.6)	.19
Median passive smoking exposure, h/d (IQR)§	1.0 (0.0–3.0)	0.5 (0.0–2.5)	.42
Earlier serious illness or operation, n (%)	8 (16)	3 (6)	.20
Taking medication, n (%)	16 (32)	14 (28)	.83
Dietary habits			
Median meat servings per month (IQR)	30 (20–30)	20 (8–30)	.02
Median fish servings per month (IQR)	3 (3–3)	8 (3–8)	.008
Median dairy servings per month (IQR)	58 (31–68)	48 (33–68)	.52
Consume local meat, n (%)	21 (48)	1 (2)	<.001
Consume local dairy products, n (%)	23 (50)	11 (23)	.01
Consume local fruit or vegetables, n (%)	29 (60)	10 (21)	<.001
Median dietary animal fat intake, g/d (IQR)	48 (35–57)	41 (30–54)	.06
Internal exposure marker			
Urinary cadmium, nmol/mmol creatinine‖	0.16 (0.13–0.19)	0.19 (0.16–0.23)	.15

* Data are mean (SD) unless stated otherwise. BMI indicates body mass index; IQR, interquartile range.
† *P* value median: Mann-Whitney *U* test; *P* value mean: *t* test; categories *P* value: Fisher exact test.
‡ Calculated for those that reported consuming alcohol.
§ Missing data for 16 participants.
‖ Urinary cadmium concentrations (geometric mean and 95% confidence interval) were corrected for smoking (current smoker, past smoker, and hours per day of passive smoking) and age.

than in Antwerp. The groups were not different in education level, residence period, alcohol consumption or smoking status, and medical history. The number of grams of alcohol intake by the alcohol consumers was similar in Peer and in Antwerp. In both regions, a similar proportion of participants took medication; more than 80% of them felt ''good'' at the time of the investigation, and none reported a ''bad'' health status (data not shown). Men from Peer consumed meat more frequently but fish less frequently. The consumption of local agricultural produce in Peer was higher for all investigated parameters. Men from Antwerp and Peer had similar levels of urinary cadmium (UCd).

UCd concentrations rose significantly by 4.2% with age after correction for past and current active or passive smoking status (95% CI, 1.8%–6.6%; $P = .001$), and this relationship was apparent at a higher level in Antwerp than in Peer (25.9% higher; 95% CI, −3.6%–64.5%; $P = .09$). Ex-smokers had slightly higher UCd (picomole per millimole [pmol/mmol] creatinine) levels than those who had never smoked (median [IQR], 187.1 [157.5–261.8] vs 158.3 [103.1–246.0]; $P = .08$). We found no association between the consumption of organ meat, fish, shrimp, or self-grown vegetables and UCd.

Semen volume and TSC but not sperm concentration correlated positively with duration of abstinence (r for semen volume, 0.35 [$P < .001$] and TSC, 0.25 [$P = .01$]). Time (minutes) between self-reported semen sample collection and first processing by a nurse was similar in both areas (GM [95% CI], 36.7 [32.2–41.9] for Peer and 36.6 [31.8–42.2] for Antwerp, respectively; $P = .98$). The delay between semen processing and laboratory investigations was significantly higher in Antwerp than in Peer (median [IQR] number of days, respectively, 5.0 [4–12] and 4 [3–7]; $P = .001$). However, this parameter did not negatively influence measured sperm concentration, semen volume, or TSC because the respective correlation coefficients were all small and nonsignificant.

E2, fE2, LH, FSH, inhibin B, and SHBG were not related to age ($r = -0.07$, $P = .51$; $r = -0.04$, $P = .67$; $r = 0.007$, $P = .95$; $r = 0.03$, $P = .79$; $r = -0.03$, $P = .80$; and $r = -0.07$, $P = .47$; respectively), in contrast to total T and fT ($r = -0.32$, $P = .001$; $r = -0.38$, $P < .001$; respectively). Former duration of smoking and self-reported hours per day of passive smoking was not correlated to any sperm or hormone parameter. The sperm and hormone values of the 2 current smokers were well within the normal range of the total population, and so no separate statistical analyses for these parameters were performed. Calculated amount of grams of alcohol consumed per day was not related to any hormone or sperm parameter. Participants with a history of serious illness or operation had a drastically lower TSC before and after correction for age, completeness of the semen sample, and abstinence period (GM [95% CI], 38.4 $\times$ 10^6/mL [20.3–73.0] vs 117.7 $\times$ 10^6/mL [95.1–145.8]; $P = .02$).

Regional Differences in Reproductive Parameters

A detailed analysis of regional differences in reproductive parameters has been published elsewhere (Dhooge et al, 2007). In short, after correction for age, self-reported completeness of semen sample, and abstinence period, lower values in Peer compared with Antwerp were seen for sperm concentration (GM [95% CI], 32.4 $\times$ 10^6/mL [24.0–43.8] vs 49.2 $\times$ 10^6/mL [36.5–66.5]; $P = .06$), TSC (GM [95% CI], 79.9 $\times$ 10^6/mL [59.1–108.0] vs 135.7 $\times$ 10^6/mL [100.4–183.4]; $P = .02$), and sperm morphology—only corrected for age—(mean [SD], 11.9% (1.0) vs 17.5% [1.0]; $P < .001$). A similar decrease was seen for FSH (GM [95% CI], 4.0 IU/L [3.5–4.7] vs 4.8 IU/L [4.2–5.6]; $P = .09$), total T (GM [95% CI], 13.3 nmol/L [12.3–14.4] vs 14.8 nmol/L [13.6–16.0]; $P = .09$), and fT (GM [95% CI], 311.3 pmol/L [289.2–335.0] vs 351.2 pmol/L [326.6–377.6]; $P = .03$) after correction for age, BMI, and time of day blood was collected. However, neither inhibin B, LH, E2, fE2, the inhibin B/FSH ratio, nor the testosterone/LH ratio were different between regions. Excluding those cases reporting a previous serious illness or operation did not influence the statistics on regional differences in fT, sperm morphology, or TSC.

Relationship Between Reproductive Parameters and Food Consumption and Cadmium Exposure

People consuming locally produced vegetables (n = 37 of 94) but not fruit had significantly lower serum fT and LH (Wilcoxon rank sum test, both $P = .04$), marginally significantly lower FSH (Wilcoxon rank sum test, $P = .05$), and nonsignificantly lower sperm morphology and sperm concentration (Wilcoxon rank sum test, $P = .13$ and $P = .27$, respectively). Local meat consumers (n = 22 of 88) had nonsignificantly lower total T, LH, and sperm morphology (Wilcoxon rank sum test: $P = .09$, .06, and .09, respectively) and significantly lower fT ($P = .007$); 87% of the participants reporting eating or not eating local vegetables also did so for local meat. In a separate question the participants were asked to quantify their monthly intake of locally produced vegetables. In the group of 86 people answering in a consistent manner on both questions, monthly consumption of such vegetables was significantly negatively related to sperm morphology, total T, fT, and LH ($r = -0.23$, $P = .04$; $r = -0.22$, $P < .05$; $r = -0.32$, $P = .02$; $r = -0.27$, $P = .01$; respectively) and less consistently to sperm concentration ($r = -0.11$, $P = .32$) but neither to TSC, inhibin B, FSH, nor the T/LH or the inh/FSH ratio. Table 2 shows sperm and hormone parameters according to the frequency of reported intake of self-grown vegetables. Scatter plots and iterative weighted least-squares Loess regression suggested a nadir in the relationship with some of the endocrine or sperm parameters at around 10 servings per month, which we further investigated by applying piecewise linear regression. Per unit increase of monthly intake of locally produced vegetables, free and total T levels, and sperm concentration declined over the whole range of the explanatory variable, whereas this was the case for LH, FSH, and sperm morphology in the range of 0–10 consumptions per month, remaining constant thereafter. Age was included as a possible confounder in the linear regression model of sperm concentration and morphology and, additionally, BMI in that of the hormone parameters (Table 3). UCd was not related to any sperm or hormone level.

Discussion

We report on lower semen quality in a rural region in Flanders compared with that in a highly industrialized city 80 km away. No regional differences in smoking history, average alcohol intake, or educational status were noted, but people in the rural area consumed local agricultural produce significantly more frequently. In addition, the frequency of intake of self-grown vegetables was significantly negatively related to a number of the investigated reproductive and endocrine parameters.

To the best of our knowledge this is only the second study set up to compare male reproductive parameters in an urban and rural area in a sample of the general population using a uniform study protocol (Swan et al, 2003a). We previously described the regional differences in semen quality and their relationship with the measured hormone levels (Dhooge et al, 2007). In short,

Table 2. *Uncorrected reproductive parameters according to self-reported intake frequency of homegrown vegetables**

	# of Servings Per Month (# of Participants)					
	0 (54)	1 (7)	3 (9)	8 (14)	20 (4)	30 (12)
Sperm concentration (× 10^6/mL)	44.0 (32.9–58.7)	55.3 (24.8–123.3)	46.3 (22.8–94.0)	43.0 (24.4–75.8)	41.8 (14.5–120.9)	17.4 (9.4–32.2)
Sperm volume (mL)	2.6 (2.1–3.1)	2.9 (1.7–4.9)	2.2 (1.4–3.6)	2.5 (1.7–3.6)	3.1 (1.5–6.3)	2.9 (1.9–4.4)
Total sperm count (× 10^6)	113.1 (84.9–150.6)	158.0 (71.3–350.3)	103.5 (51.3–208.9)	106.7 (60.8–187.4)	129.7 (45.2–372.0)	51.0 (27.8–93.7)
Sperm morphology (%)	14.0 (12.0–16.4)	16.7 (10.4–26.7)	12.5 (8.5–18.3)	8.2 (6.0–11.1)	12.5 (7.0–22.2)	15.1 (10.3–22.1)
Hormone values						
Inhibin B (ng/L)	218.0 (202.2–235.1)	236.5 (191.9–291.5)	223.0 (185.5–268.2)	234.4 (202.2–271.7)	216.8 (164.4–285.9)	207.8 (177.1–243.8)
FSH (IU/L)	4.7 (4.1–5.5)	3.6 (2.5–5.4)	4.1 (2.9–5.8)	3.7 (2.8–4.9)	4.4 (2.6–7.3)	4.5 (3.3–6.0)
LH (IU/L)	4.5 (4.1–5.0)	2.5 (1.9–3.3)	5.3 (4.1–6.8)	3.5 (2.9–4.2)	4.3 (3.0–6.2)	3.6 (2.9–4.4)
Testosterone (nmol/L)	14.9 (13.6–16.3)	14.1 (11.0–18.1)	12.9 (10.3–16.0)	12.4 (10.4–14.8)	13.7 (9.9–19.1)	13.1 (10.9–15.9)
Free testosterone (nmol/L)	355.1 (327.6–384.9)	342.1 (273.6–427.9)	312.8 (256.8–381.0)	290.8 (248.3–340.6)	317.7 (236.3–427.1)	287.9 (242.7–341.5)

* Data are geometric mean (95% confidence interval); FSH indicates follicle-stimulating hormone; LH, luteinizing hormone.

Table 3. *Dose-effect relationships between food intake and reproductive parameters in men**

	% Decrease	95% Confidence Interval	*P*
Effects associated with a unit increase in monthly vegetable consumption in the whole population			
Sperm concentration (× 10^6/mL)†	2.8	0.7–4.8	.009
Total sperm count†	2.3	0.3–4.4	.03
Sperm morphology (% normal forms)‡	6.8	2.9–10.6	.001
Testosterone (nmol/L)†	0.6	0.0–1.1	.05
Free testosterone (pmol/L)†	0.7	0.2–1.3	.008
LH‡	2.2	−0.7–5.0	.13
FSH‡	2.6	−1.0–6.2	.15
Effects associated with a unit increase in monthly vegetable consumption in the reduced population§			
Sperm concentration (× 10^6/mL)†	2.3	0.1–4.5	.04
Total sperm count†	1.9	0.3–4.0	.10
Sperm morphology (% normal forms)‡	7.0	2.8–11.0	.002
Testosterone (nmol/L)†	0.6	−0.1–1.2	.07
Free testosterone (pmol/L)†	0.7	0.2–1.3	.01
LH‡	3.6	0.6–6.5	.02
FSH‡	3.5	−0.4–7.2	.08

* Sperm parameters adjusted for age; hormone values adjusted for age and body mass index. LH indicates luteinizing hormone; FSH, follicle-stimulating hormone.

† Percentage change in the range of 0–30 consumptions per month.

‡ Percentage change in the range of 0–10 consumptions per month, remaining constant thereafter.

§ Only those men answering in a consistent manner on both questions regarding their intake of locally produced vegetables were included (n = 86).

sperm concentration, TSC, and sperm morphology were more than 30% lower in Peer than in Antwerp, but this was not accompanied by lower inhibin B levels. The testicular polypeptide inhibin B has been shown to reflect successful sperm development up to the level of the round spermatids, both in testes with normal spermatogenesis and in those with spermatogenic arrest (Andersson et al, 1998; Marchetti et al, 2003). In this respect, partial spermatogenic arrest (PSA) at or beyond the stage of meiosis could lead to decreased sperm output without significantly affecting inhibin B levels, as has been argued before (Andersson et al, 1998; von Eckardstein et al, 1999). In addition, the T/LH and inh/FSH ratios (Andersson et al, 2004a, 2004b), both markers of testicular fitness, were similar in both cohorts of the present study. This, in combination with the observed 17% reduced FSH levels in the men from Peer, supports a theoretical paradigm of a PSA at the spermatid level related to hypophysal insufficiency (Martin-du Pan and Campana, 1993) rather than

a testicular factor as the cause of the observed lower reproductive status in the rural area.

We could not ascertain whether this decreased gonadostat function has its origin in fetal life and thus would be a physiological mechanism in addition to the recently defined TDS (Skakkebaek et al, 2001). Eighty-six percent of the participants living in Peer were born in a rural area, whereas 92% of the participants from Antwerp were born in a city (data not shown). We did not acquire information regarding the participants' birth weight or the medical treatment or lifestyle of the participants' mothers during pregnancy (Jensen et al, 2004).

Alternatively, present-day lifestyle or environmental factors might be equally plausible etiologic factors in the observed regional differences (Martin-du Pan and Campana, 1993; Vermeulen, 1993; Pajarinen and Karhunen, 1994). Conflicting evidence exists on the influence of smoking on sperm parameters (Chia et al, 1994; Sergerie et al, 2000; Chen et al, 2004), but moderate alcohol consumption has been related to PSA (Pajarinen et al, 1996). People from the rural area were not different from those in the urban area with regard to their self-reported (low) alcohol consumption, smoking status, or history, and neither parameter was related to any sperm or hormone parameter (data not shown). Chronic illness has been associated with lowered testosterone levels (Gray et al, 1991), and we found people who reported a previous serious illness or operation to have drastically decreased TSC. However, no regional difference in reported health status and history was noted, and taking medication had no effect on any reproductive parameter (data not shown).

We found the participants from the rural area to consume locally produced food products significantly more often than those from the urban region. Individuals who reported eating homegrown vegetables had lower fT, LH, and FSH. We separately assessed the number of monthly consumptions of these vegetables and, in those individuals responding in a consistent manner on both questions, intake frequency significantly negatively correlated with sperm morphology and the hormones T, fT, and LH. A general linear model largely confirmed these relationships, even after inclusion of possibly important confounding variables. Juhler et al (1999) found that the percentage of sperm with normal morphology evaluated according to the strict criteria, but not sperm concentration, the testosterone/SHBG ratio, LH, FSH, or inhibin B, increased with the proportion of organically grown fruit or vegetables in the farmers' diets. Members of organic food associations in Denmark had higher sperm concentrations but not a higher proportion of sperm with normal morphology compared with an allegedly unexposed control group of employees of an airline company (Jensen et al, 1996). A diet exclusively based on organically produced food had no effect on the mean percentage of sperm anomalies in fertile men from 4 European cities (Auger et al, 2001).

Notwithstanding reductions in Europe during the 1990s, cadmium production, consumption, and emissions to the environment have increased dramatically during the 20th century (Jarup et al, 1998). Industrial cadmium emissions and the application of phosphate fertilizers or sewage sludge to farmland may lead to contamination of soils and to increased cadmium uptake, especially with low soil pH, by crops and vegetables grown for human consumption (Jarup, 2003). In the past years, several studies have investigated possible cadmium-related health effects in people from a number of regions in Flanders, including the Antwerp area, because of a documented historical contamination with this heavy metal (Staessen et al, 1996; Nawrot et al, 2006). UCd is a measure of the body's cadmium burden, in contrast to blood levels, which generally reflect recent exposure (Jarup et al, 1998). In accordance, in the present study age was a significant determinant of UCd. The UCd levels found in this study group were close to the levels found in the adolescents but substantially lower than the ones detected in the older women, which were all investigated in the framework of the FLEHS (Staessen et al, 2001; van Larebeke et al, 2004). In the latter cohort UCd levels correlated significantly with the number of monthly consumptions of self-grown vegetables ($r = 0.24$, $P = .001$; $r = 0.21$, $P = .003$; respectively; W.D., unpublished data, 2000) but not in the adolescents or the males. A previous study from Flanders described urinary but not blood cadmium levels to correlate with cadmium levels in vegetables from the participants' kitchen gardens (Staessen et al, 1994). Average UCd levels were, however, at the low end of levels found in other countries (Jarup et al, 1998; Department of Health and Human Services, 2003; Yassin and Martonik, 2004). Further, the urinary concentrations in this study suggest a daily intake well below the provisional tolerable weekly intake (PTWI) (Satarug and Moore, 2004).

Cadmium is one of the well-known reproductive toxicants in experimental animals, but the effects on human fertility are less conclusive (Jarup et al, 1998; Benoff et al, 2000). In an infertility clinic setup, some studies report lower blood or seminal plasma cadmium concentrations in fertile compared with infertile patients (Seren et al, 2002), whereas others do not find such differences (Keck et al, 1995). Recently, cadmium exposure was related to decreased sperm concentration and TSC in a nonsmoking, nonoccupationally exposed population and to an increased proportion of sperm

with abnormal morphology in industrial workers (Telisman et al, 2000; Xu et al, 2003). In the present study we found no relationship between the body burden of cadmium and sperm parameters or the sex hormones FSH, LH, or T. Altogether, the lack of associations of UCd with the number of monthly consumptions of self-grown vegetables with the hormone and with the sperm parameters strongly suggests that cadmium was not the factor present in the vegetables that contributed to the reduced reproductive parameters in our male population.

Rural activities are intuitively associated with pesticide exposure. The limited available data from the Flemish Environment Agency (2005) suggest pesticide contamination in the immediate surroundings of the investigated rural area, as is described in ''Methods.'' Stehr-Green et al (1988) concluded that at least some of the pesticide-related body burden in a rural-dwelling population is likely attributable to exposures through consumption of homegrown food products. Children consuming organic fruits and vegetables had significantly lower urinary organophosphorus pesticide metabolite levels compared with children with a conventional diet (Curl et al, 2003). Swan et al (2003a) reported on lower sperm concentration and motility but not morphology in 4 US cities, one of which was proximate to intensive agriculture, and concluded that the former 2 sperm quality measures might be reduced in semirural and agricultural compared with more urban areas. In a subsequent study they investigated urinary pesticide metabolite levels in 86 men from 2 areas with a different pesticide application profile and found significant linear relationships between the levels of current-use pesticides and sperm morphology only in the rural cohort. Similar but weaker associations were seen with sperm concentration (Swan et al, 2003b).

In line with the above-described regional differences, the decrease in reproductive parameters with a higher consumption of self-grown vegetables suggests impairment of the hypothalamic-pituitary functional unit rather than of the testicular compartment. Rat in vivo data show that formamidines can acutely affect the neuroendocrine control of the preovulatory LH surge in females and reduce serum LH levels in the male rat. However, in the less sensitive males, adaptive processes do seem to take place resulting in a normalization of hormone values after prolonged exposure (Cooper et al, 1999). The organophosphate quinalphos has been described to decrease spermatogenesis in adult rats, with the effects on serum FSH, LH, and testosterone being far less consistent (Ray et al, 1992; Sarkar et al, 2000). In humans, pesticides have been described to cause unaltered, decreased as well as increased secretion of gonadotropins, but the latter scenario is more probably the consequence of impaired testosterone synthesis through Leydig cell dysfunction (Hanke and Jurewicz, 2004; Kamijima et al, 2004). Overall, no clear picture of hormone disruption by occupational exposure to pesticides emerges from the limited available epidemiologic data (Larsen et al, 1999; Abell et al, 2000; Ayotte et al, 2001).

Although rather precise quantitative data on the differential use of pesticide types in the agricultural and nonagricultural sector are available in Belgium (De Smet et al, 2005), more detailed information on the application patterns of pesticides on homegrown vegetables or fruit is not. We did not measure pesticides in the vegetables consumed, nor was our questionnaire designed to investigate the possible types of pesticide exposure. Nevertheless, the experimental and epidemiologic data are inconclusive regarding effects of pesticides at the level of the gonadostat that were seen in this study.

Overall, in the present study, surprisingly low semen values were found compared with cohorts from other European studies (Jørgensen et al, 2002; Richthoff et al, 2002; Slama et al, 2002). Although not unusual for this type of study, an initial overall response rate of 30% may seem low, and thus our study is at risk for selection bias. Indeed, the comparability of the present population to the above-described European cohorts, or its representativeness for the general Flemish population, is limited, as discussed previously (Dhooge et al, 2007).

Seasonal differences in sperm quality measures have been previously described. For logistics reasons our study took place at separate periods in summer. The participants from Antwerp, with the overall better sperm characteristics, were evaluated in late June and early July, whereas those in Peer were sampled in the first 2 weeks of September. Gyllenborg et al (1999) did not measure sperm morphology but found TSC and sperm concentration to be lower in June/July compared with August/September, which is opposite to our results. Others have evaluated the effect of season on sperm morphology. Chen et al (2003) found sperm morphology to be lower in spring and summer than in fall and winter. In a large study encompassing partners of pregnant women from 4 European cities, no seasonal variation in the percentage of morphologically normal sperm was found. However, the mean number of sperm defects per abnormal spermatozoa was higher during spring than during autumn and winter (Auger et al, 2001). Together these data suggest that the area differences in sperm parameters we registered in the present study cannot be explained by a seasonal factor. In addition, we have previously argued that older age and higher BMI (Table 1) were unlikely to have caused the lower semen and hormone values of the population

recruited from the rural area (Dhooge et al, 2007). Nevertheless, even if confounding factors have contributed to the regional differences in male reproductive measures, this does not alter the main conclusion of this study (ie, that these differences seemed to be related to the consumption of vegetables from the family's own garden).

The present hypothesis generating results leaves a number of questions unanswered. Further studies should investigate in more detail the diet of the investigated cohorts. Particularly important would be to evaluate the type of vegetables that were consumed and whether the presence of xenobiotic substances in or on the vegetables could provide a plausible explanation for the observed lower reproductive status of the rural cohort.

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