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Differences in *HPRT* mutant frequency among middle-aged Flemish women in association with area of residence and blood lead levels

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Biomarkers were measured in residents of Wilrijk and Hoboken, industrial suburbs of the city of Antwerp, and of Peer, a rural municipality in Flanders, Belgium. Persons with known occupational exposures to toxic compounds or commuting over long distances were excluded. Here, we report the hypoxanthine phosphoribosyltransferase gene (*HPRT*) variant frequencies for 99 non-smoking women aged 50–65 years. *HPRT* values above the detection limit (V_{fpos} values) were observed for 43 subjects (21 from Peer, 22 from Antwerp). The median (10th to 90th percentiles) *HPRT* variant frequency (V_{fpos}) in peripheral lymphocytes was 9.59 (3.44–56.99) for Peer and 3.57 (1.57–13.96) for Antwerp. The V_{fpos} value was significantly higher in Peer than in Antwerp, both in terms of crude data ($p = 0.011$) and after correction for age, level of education, smoking status, serum level of selenium and body mass index through analysis of covariance ($p = 0.011$). For the total study population, serum lead concentration showed a non-significant positive correlation with $\ln V_{fpos}$. In addition, subjects with a blood lead concentration above the median tended to have higher V_{fpos} values (9.45×10^{-6} for 'high' group versus 5.21×10^{-6} for 'low' group; $p = 0.077$ after correction for confounding). Subjects with a serum selenium level above the median tended to have lower V_{fpos} values (4.99×10^{-6} for 'high' group versus 9.83×10^{-6} for 'low' group; $p = 0.051$ after correction for confounding). These data are consistent with an indirect genotoxic effect of lead and with an antimutagenic effect of selenium.

Keywords: mutant frequency, *HPRT*, environment, lead, selenium, pollution.

Introduction

The Flemish Environment and Health Study (FLEHS) was a feasibility study on the use of biomarkers to evaluate whether residence in areas with differing pollution pressure had a significant impact on internal exposure to pollutants and

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resulted in adverse biological and health effects. Both internal exposure and effect biomarkers were measured, on an individual basis, in residents of Wilrijk and Hoboken, industrial suburbs of the city of Antwerp, and of Peer, a rural municipality in Flanders, the Dutch-speaking northern part of Belgium. As shown in table 1, Wilrijk and Hoboken contain important sources of pollution, including a large non-ferrous smelter in Hoboken, which has caused important lead pollution in the past, and two waste incinerators in Wilrijk. In contrast, Peer is a rural municipality containing mainly agriculture. Participants were asked to fill in questionnaires on dietary habits, education, reproductive history, residence history, smoking and alcohol intake.

Table 1. Characteristics of the study areas.

	Wilrijk[a] (suburb of Antwerp)	Hoboken[a] (suburb of Antwerp)	Peer[b]
Inhabitants (number/km^2)	2253	2253	168
Sources of pollution	Metallurgic industry	Large non-ferrous smelter	Mainly agriculture
	2 waste incinerators	Historical pollution by lead	Military airbase in the North
	Crematorium	Metallurgic industry	Few industrial activities
	Plastics industry	Printer	
	Printer	Electronic equipment	
	Highway > 80 000 vehicles/day	Highway > 80 000 vehicles/day	
Air pollutants			
Mean SO_2 concentration per day (1997)[c]	20–60 μg m^{-3}	20–60 μg m^{-3}	11–NA μg m^{-3}
Mean tetrachloroethylene concentration per year (1997)	1.2 μg m^{-3}	1.2 μg m^{-3}	0.2/0.4 μg m^{-3} (Maasmechelen, two measuring points)
Mean ozone concentration per 8 h[c]			
1997	20–102 μg m^{-3}	20–102 μg m^{-3}	NA
1998	26–NA μg m^{-3}	26–NA μg m^{-3}	38–NA μg m^{-3}
Mean lead concentration of suspended particles (1997)[d]	NA	0.08–1.35 μg m^{-3}	0.05 μg m^{-3} (Hechtel-Eksel)[e]
Mean B[*a*]P concentration per year (in Total Suspended Particles) (1996)	0.8 ng m^{-3}	0.8 ng m^{-3}	0.31 ng m^{-3} (Gellik)
Precipitation of pollutants			
Dioxin (1993–1997)[d]	3.6–13 pg TEQ m^{-2} day^{-1}	10.7–30 pg TEQ m^{-2} day^{-1}	3.1–8.0 pg TEQ m^{-2} day^{-1} (Hechtel-Eksel)[e]

NA, not available; B[*a*]P, benzo[a]pyrene.

[a] When separate data for Wilrijk or Hoboken were not available, data for Antwerp as a whole are given.

[b] When data for Peer were not available, data for a neighbouring municipality are given.

[c] 50th to 98th percentile.

[d] Minimum to maximum (if several measuring points given).

[e] Hechtel-Eksel is part of the Peer area.

Results concerning renal toxicity, sexual maturation and the immune system in adolescents have been recently published (Staessen *et al.* 2001, Nawrot *et al.* 2002, Van Den Heuvel *et al.* 2002).

Here we report the measurement of hypoxanthine phosphoribosyltransferase gene (*HPRT*) mutant frequency in peripheral blood lymphocytes obtained from 99 non-smoking women aged 50–65 years resident in the three areas studied (47 from Peer, 23 from Wilrijk, and 29 from Hoboken). Mutations in the *HPRT* gene are easily detectable in lymphocytes from exposed subjects. They are not necessarily involved in the process of carcinogenesis, but are considered to reflect mutational events in genes that are critical for this process. The observations on biomarkers of internal exposure will be published in detail in a separate paper, but some data are mentioned in this article.

Middle-aged women were chosen because of the accumulation in the body of certain pollutants with age, and because of the fact that in Flanders many women of that age category spent most of their life residing in the same region and working at home without commuting over long distances. Exclusion criteria for enrolment in the study were occupational exposures, working in a region with environmental characteristics clearly different from the area of residence, and commuting over long distances. Much attention was paid to possible confounding effects by life style and personal characteristics.

Subjects, materials and methods

Selection of study areas

Hoboken and Wilrijk, industrial suburbs of the city of Antwerp (404 241 inhabitants), are located 11–13 km south-east from the chemical and petrochemical industry established in the seaport of Antwerp. Peer (14 622 inhabitants) is situated in a rural area, 70 km east of Antwerp, and was chosen as the 'control' area in spite of its intensive agriculture. Sources of pollution and some other characteristics of these municipalities are given in table 1. The median value of the mean daily respirable particle (PM10) concentration in Antwerp was 26 $\mu g\,m^{-3}$ in 1997; no data were available for Peer. Surface water quality was substantially better in Peer than in Antwerp. The two waste incinerators in Wilrijk have been in operation from 1971 and 1980, respectively. In 1997, they had annual turnovers of 23 000 and 110 000 tons. They were shut down in November 1997, because the emissions of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) had exceeded the limit of 0.1 ng international toxic equivalent (I-TEQ) per Nm^3. In the past, emissions of PCDD/PCDFs (annual measurement) were between 2 and 7 ng I-TEQ Nm^{-3}. In 1998, concentrations of PCDD/PCDFs in soil samples (0–5 cm depth) close to the incinerators ranged between 3.5 and 35.9 ng I-TEQ kg^{-1} dry weight (De Fré *et al.* 1999). Although there is a military airfield in the north of the municipality and some polluting industries at a distance of about 15 km, measured environmental pollution was lower in Peer than in many other places in Flanders, at least for the few parameters for which data were available (table 1).

Selection of the study population

The initial selection comprised 2898 women aged between 50 and 65 years, selected randomly from the population registers of the municipalities under study. The women were contacted by letter. About half of the 40.1% and 30.8% responders in Antwerp (Hoboken plus Wilrijk) and Peer, respectively, were further selected ($n = 685$) after consideration of the exclusion criteria, which were present smokers, residing in the study area for less than 10 years, 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. Of the selected women, 255 were contacted by telephone and 200 individuals agreed to participate in the study. *HPRT* mutant frequency was measured in peripheral blood samples from a randomly chosen subset of 99 women (47 from Peer, 23 from Wilrijk and 29 from Hoboken). All participants gave their informed consent. The ethics committee of the University of Leuven approved the study. Throughout the study a communication plan (with the participating subjects and local authorities)

was in force. Each participant was offered the opportunity to have an interview with a physician-researcher working on the study.

Measured parameters

Height and body weight were measured by nurses working for the project, who also collected about 200 ml of urine and 40 ml of blood from each subject. Blood samples were collected in polyethylene containers. Immediately after sampling, the serum was separated. Split samples of serum, plasma, whole blood and urine were stored at 4°C or immediately deep frozen. All laboratory analyses were performed in specialized laboratories that met national and international quality-control standards and were performed blindly, without the laboratories having information on the subjects. For each participant, a series of routine haematological parameters and serum concentrations of total fat, triglycerides, cholesterol, selenium, vitamin A, vitamin E, zinc and copper were determined. The following biomarkers of exposure were measured in serum: dioxin-like activity, measured using the calux bioassay based on *in vitro* activation of the aryl hydrocarbon receptor (AhR) of cultured H4IIE cells, as described by Koppen *et al.* (2002); PCB 138, PCB 153 and PCB 180 (indicator polychlorinated biphenyls [PCBs]), measured using a high-resolution gas chromatograph with electron capture detection equipped with two capillary columns of different polarity, as described by Koppen *et al.* (2002); cadmium (in urine and blood) and lead (in blood), measured by electrothermal atomic absorption spectrometry using the stabilized temperature platform furnace method coupled with a Zeeman-effect background correction system, as described by Claeys *et al.* (1992); and 1-hydroxypyrene, measured in urine after enzymatic hydrolysis and clean-up through solid phase extraction, using high performance liquid chromatography and fluorimetric detection, as described by Van Hummelen *et al.* (1993). Urinary measurements were standardized to 1 mmol of creatinine. The complete results of the measurements of biochemical parameters and biomarkers of exposure will be published in the future by Van Loon *et al.*; those concerning dioxins and PCBs were published by Koppen *et al.* (2001) and Covaci *et al.* (2001).

HPRT mutant frequency

Blood samples for the measurement of *HPRT* mutant frequency were brought to the laboratory without delay. As many different analyses were performed on the blood sample from each subject, only 5 ml of blood was available for the *HPRT* test. The *HPRT* mutant frequency was determined in terms of *HPRT* variant frequency by the method of Zwingmann *et al.* (1998), using immunocytochemical staining with monoclonal anti-5-bromodeoxyuridine antibodies (anti-BrdU). Briefly, a 5 ml sample of heparinized blood was diluted (1:10, v/v) in RPMI 1640 without supplements and stored for 24 h at 4°C to reduce the effect of spontaneously cycling lymphocytes. After this cold storage, cells were diluted at a density equal to 0.4 ml whole blood in 5.0 ml complete medium plus 0.2 ml phytohaemagglutinin. 6-Thioguanine (TG) was added (final concentration 2×10^{-4} M) and after 24 h the cells were labelled with 2.5×10^{-6} M BrdU for 16 h. Two of the 10 5 ml cultures were used to determine the labelling index (LI_c), calculated as the number of labelled nuclei divided by the total number of nuclei evaluated. Eight 5 ml cultures were used for TG cell selection. For these TG cultures, at the end of the incubation period, after the hypotonic treatment and fixation, all cells were transferred to slides on which a small area was demarcated with stearine, and the total volume of cell suspension was determined. In 1 μl of a 1:10 dilution of this suspension, the number of nuclei transferred to TG slides was counted after Giemsa staining. The slides of the TG cultures were not immediately counterstained but first screened for brown BrdU-containing cells. Next, the TG slides were counterstained with Giemsa to allow a complete view of all nuclei. Slides were scored by one well-trained observer. The labelling index of the TG cultures (LI_t) was determined by the number of brown nuclei on the TG slides divided by the total number of nuclei on the TG slides. The variant frequency (V_f) of the TG nuclei was defined as LI_t/LI_c. When LI_t was zero, V_f was assumed to be equal to the detection limit in that measurement (e.g. one BrdU-positive nucleus). The subset of V_f values after exclusion of persons for whom no variant cells were actually observed gave the parameter V_{fpos}. In this way, the variant frequency parameter V_{fpos} relates only to the persons for which the variant frequency measurement gave a result above the detection limit.

Data from questionnaire

All participants completed a self-reporting questionnaire on education, residence history, food intake, smoking and alcohol consumption. With regard to education, taken as a measure of social status, participants were classified according to the most advanced form of education they had received: primary school or the first three years of secondary school, complete secondary school, or higher education. With regard to smoking status, participants were classified as ex-smokers if they had smoked at least one cigarette a day for at least 1 year; otherwise they were classified as never-smokers. Data on passive smoking were available for only 53 of the participants for whom *HPRT* variant frequency was measured and were therefore not used in most of the analyses presented below. The following food-intake parameters, calculated from the semiquantitative food frequency questionnaires on dietary habits over

the last year, were used: ethanol intake (g per day); frequency of consumption of meat or organ meat'meat or organ meat' Is the move OK?. (smoked, salted, grilled, roasted or baked) per month; frequency of consumption of baked, roasted or grilled meat per month; frequency of consumption of fish per month; frequency of consumption of smoked or salted meat or fish per month; frequency of consumption of fish, organ meat, mussels or shrimps per month; total intake of animal fat (g per day); frequency of consumption of dairy products per day; total intake of calcium (mg per day); number of different types of locally grown food items regularly consumed (index for consumption of local food); and frequency of consumption per month of vegetables from their own garden. In addition, the following lifestyle parameters were calculated from the questionnaires: total number of pregnancies, and total number of weeks spent breastfeeding.

Statistical analyses

As the distribution of the variant frequency parameters V_f and V_{fpos} was not normal, their natural logarithms $\ln V_f$ and $\ln V_{fpos}$ were used in t-tests and for analysis of covariance (ANCOVA). The statistical significance of differences between crude data was tested using a two-tailed t-test. Correction for confounding parameters was performed using ANCOVA, limited to main effects only. After correction using ANCOVA, geometric mean values are given. To select relevant parameters that might contribute to some extent to differences in *HPRT* mutant frequency, stepwise regression was performed with F to include = 3 and F to exclude = 2. Simple linear regression, stepwise regression, multiple regression, t-tests (always two-tailed), ANCOVA, χ^2 tests and non-parametric Mann–Whitney U-tests were performed using Statview 5.0.1 or Statistica programs.

Results

Characteristics of participants, food intake and internal exposure

Table 2 gives an overview of some of the recorded characteristics of the participants, including physical characteristics, education, smoking status, passive smoking, and reproductive history. Potentially important differences between residents of Antwerp and Peer were noted regarding body height and serum triglyceride concentration, the percentage of participants whose education was limited to primary school or the first three years of secondary school, passive smoking, and the number of pregnancies and the number of weeks spent breastfeeding.

Table 2. Physical characteristics, education, smoking status, passive smoking and reproductive history of study participants.

Characteristics	Antwerp ($n = 52$)	Peer ($n = 46$)	p
Age (years)[a]	57.5 (51.7–63.0)	58.5 (53.0–64.0)	0.17[c]
Height (cm)[b]	159.7 (5.7)	157.0 (6.4)	0.0099[c]
BMI (kg m^{-2})[a]	24.9 (21.2–33.6)	25.1 (22.0–32.4)	0.51[c]
Triglycerides (mmol l^{-1})[a]	1.33 (0.85–2.17)	1.60 (1.08–3.35)	0.033[c]
Education limited to primary school or the first 3 years of secondary school (%)	55.8	71.7	0.10[d]
Ex-smokers (%)	7.7	4.3	0.49[d]
Passive smoking (h day^{-1})[a]	0.5 (0.0–14.2) Mean 3.3	0.0 (0.0–2.0) Mean 1.0	0.068[c]
Number of pregnancies[a]	2 (0–5)	3 (0–6)	0.021[c]
Number of weeks' breastfeeding[a]	0 (0–31) Mean 10	13 (0–58) Mean 23	0.0006[c]

[a] Values are the median (10th to 90th percentile). When the median is zero, arithmetic means are also given.
[b] Values are the arithmetic mean (SD).
[c] Non-parametric Mann–Whitney U-test.
[d] χ^2 test.

Table 3. Food intake by study participants.

	Antwerp	Peer	p^a
Ethanol intake (g day^{-1})	0.0 (0.0–28.8) Mean 8.2	0.0 (0.0–16.1) Mean 5.8	0.67
Frequency of consumption of meat or organ meat (smoked, salted, grilled, roasted or baked) per month	20.5 (3.1–40.3)	20.0 (5.0–34.9)	0.44
Frequency of consumption of fish per month	8.0 (2.7–16.0)	8.0 (1.0–11.0)	0.24
Total intake of animal fat (g day^{-1})	26.8 (9.0–52.6)	24.6 (9.0–30.6)	0.19
Frequency of consumption of dairy products per day	1.6 (0.7–3.4)	1.5 (0.6–2.3)	0.28
Frequency of consumption of vegetables from own garden per month	0.0 (0.0–8.0) Mean 3.8	20.0 (0.0–30.0) Mean 19.9	< 0.0001

Values are the median (10th to 90th percentile). When the median is zero, arithmetic means are also given.

[a] Non-parametric Mann–Whitney *U*-test.

Table 3 gives an overview of some of the food intake data. Significant differences between residents of Antwerp and Peer were observed chiefly in the frequency of consumption of vegetables from their own garden and in the number of different types of locally grown food items regularly consumed (higher in Peer, data not shown).

Table 4 shows some of the internal exposure data (concentrations in serum or urine). Residents from Antwerp showed significantly higher zinc, PCB and selenium levels in their serum, and significantly lower 1-hydroxypyrene levels in their urine compared with residents from Peer.

Association of HPRT mutant frequencies with place of residence: crude data

The main crude data related to *HPRT* mutant frequency measurements in the study population are summarized in table 5. A *t*-test applied to these crude data

Table 4. Internal exposure (blood, serum or urine) of study participants.

	Antwerp	Peer	p^a
Concentration in blood or serum			
Cadmium (nmol l^{-1})	5.34 (3.56–9.43)	6.23 (4.45–10.68)	0.068
Lead (nmol l^{-1})	159.3 (85.4–245.6)	142.4 (78.2–251.0)	0.37
Zinc (μmol l^{-1})	10.25 (8.83–11.92)	9.33 (7.50–11.02)	0.0004
Dioxin-like activity (pg TEQ g^{-1} blood fat)	49.79 (8.72–90.41)	57.9 (24.9–80.9)	0.56
Sum of PCB 138, 153 and 180 (ng g^{-1} blood fat)	427.4 (257.8–660.1)	365 (262–556)	0.033
Selenium (μmol l^{-1})	1.23 (0.93–1.49)	1.09 (0.86–1.29)	0.0005
Concentration in urine			
Cadmium (nmol mmol^{-1} creatinine)	0.69 (0.40–1.59)	0.89 (0.43–1.93)	0.20
Hydroxypyrene (pmol mmol^{-1} creatinine)	40.5 (15.0–87.4)	72.4 (14.0–200.9)	0.0093

Values are the median (10th to 90th percentile).

[a] Non-parametric Mann–Whitney *U*-test.

Table 5. The LI_c of unselected cells; the total number of cells screened for variant characteristics; and V_f and V_{fpos} values according to area (crude data).

	Antwerp ($n = 52$ for V_f; $n = 22$ for V_{fpos})	Peer[a] ($n = 46$ for V_f; $n = 20$ for V_{fpos})
Total number of cells screened ($\times 10^6$)	1.52 (0.62–2.98)	1.47 (0.95–2.81)
LI_c	0.170 (0.101–0.249)	0.123 (0.058–0.220)
V_f ($\times 10^{-6}$)	4.55 (1.68–16.29) Mean 4.75	5.94 (2.39–27.06) Mean 7.25
V_{fpos} ($\times 10^{-6}$)[b]	3.57 (1.57–13.96) Mean 4.58	9.59 (3.44–56.99) Mean 11.56

Values are the median (10th to 90th percentile); the geometric mean is also given for some parameters.

[a] One participant from Peer had a very high V_f value (differing from the mean by more than 3 $\times$ SD), probably a clone, and was excluded from the statistical analysis.

[b] The parameter V_{fpos}, obtained after exclusion of persons for whom no variant cells were actually observed, relates only to the persons for which the variant frequency measurement gave a result above the detection limit.

shows that residents from Peer had significantly higher *HPRT* mutant frequencies than residents from Antwerp in terms of V_f ($p = 0.049$) and V_{fpos} ($p = 0.011$). In addition, residents from Peer had a significantly lower labelling index than residents from Antwerp ($p = 0.0027$).

Association of HPRT mutant frequencies with personal and lifestyle parameters

When variant frequency parameters V_f and V_{fpos} were plotted against age, body mass index (BMI), total number of pregnancies, total number of weeks spent breast feeding, food-intake parameters (with the exception of those relating to the consumption of local food), or serum levels of fat, triglycerides, cholesterol, vitamin A or vitamin E, no clear trend could be visualized, except for a slight trend towards higher V_{fpos} values with increasing BMI. Simple linear regression analysis and multiple regression analysis with these personal and lifestyle parameters and with all available parameters of internal exposure showed that none of these personal and lifestyle parameters was significantly correlated with the $\ln V_f$. The BMI showed a weak positive correlation with $\ln V_{fpos}$ on simple linear regression ($R^2 = 0.071$, $p = 0.088$), which was weaker still on multiple regression (squared semipartial correlation = 0.034, $p = 0.29$). To select parameters associated with differences in *HPRT* mutant frequencies for inclusion in ANCOVA analyses, a stepwise regression of the above-mentioned personal and lifestyle parameters and of serum levels of vitamin A, vitamin E and selenium, with $\ln V_f$ or $\ln V_{fpos}$ as the dependent variable, was performed; with $\ln V_f$ an F value of at least 3 was observed only for selenium serum level, and with $\ln V_{fpos}$ an F value of at least 3 was observed only for BMI.

Association of HPRT mutant frequencies with place of residence

After correction for relevant confounding parameters (age, level of education, smoking status, serum level of selenium and BMI), women residing in Peer had higher adjusted variant frequency values than women residing in Antwerp; the difference was significant for the parameter V_{fpos} (10.36×10^{-6} for Peer versus

5.11×10^{-6} for Antwerp, $p = 0.011$), while the parameter V_f showed the same trend (6.69×10^{-6} for Peer versus 5.27×10^{-6} for Antwerp, $p = 0.063$).

Association of HPRT mutant frequencies with levels of biomarkers of internal exposure

For the whole study population, variant frequency parameters V_f and V_{fpos} were plotted against serum concentrations of selenium, zinc and copper, against the sum of the serum concentrations of PCB 138, 153 and 180, against blood concentrations of cadmium and lead, and against urinary concentrations of cadmium and 1-hydroxypyrene. Only blood lead and serum selenium showed a clear trend with both V_f and V_{fpos}. As seen in figure 1, on simple linear regression analysis the concentration of lead in blood showed a positive correlation with $\ln V_f$ ($R^2 = 0.052$)

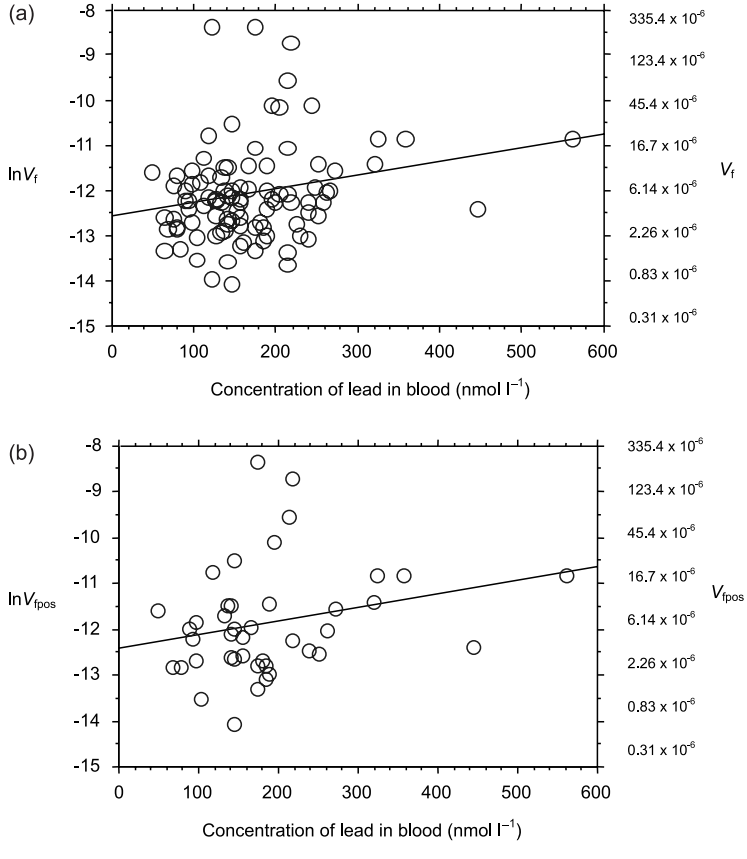

Figure 1. Regression plot of the natural logarithm of variant frequency parameters against the concentration of lead in blood. The natural logarithm (ln) is indicated on the left-hand axes, with the corresponding variant frequency levels on the right-hand axes. (a) Relationship between blood lead concentration and $\ln V_f$. On single regression analysis, regression coefficient = 0.0031 (95% confidence interval 0.00048–0.0057), $R^2 = 0.052$ ($p = 0.024$, $n = 98$). (b) Relationship between blood lead concentration and $\ln V_{fpos}$. This parameter relates only to the subjects for which the variant frequency measurement gave a result above the detection limit in the corresponding experiment. On single regression analysis, regression coefficient = 0.0029 (95% confidence interval -0.0007–0.0036), $R^2 = 0.060$ ($p = 0.12$, $n = 42$).

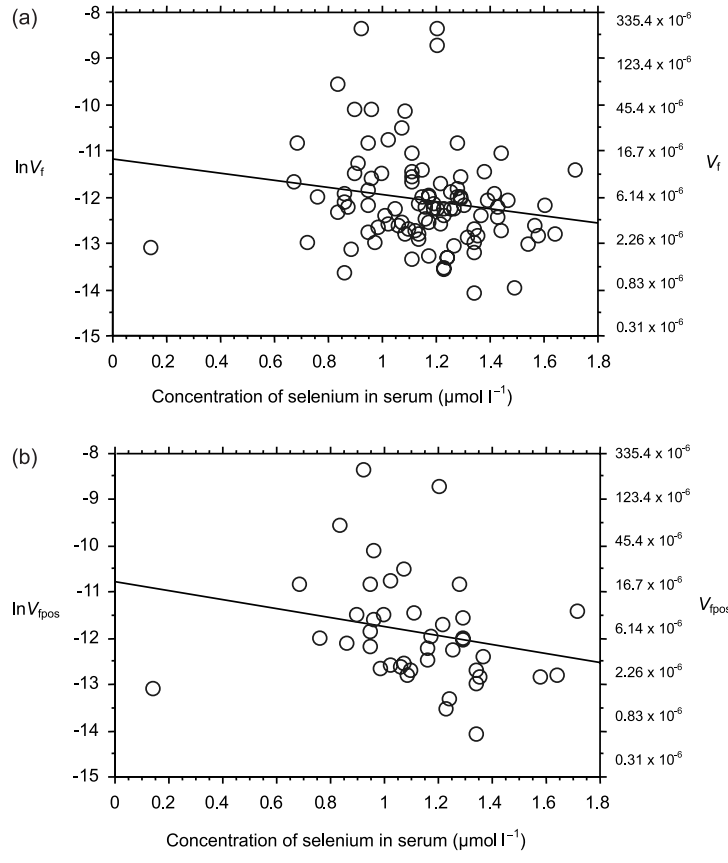

Figure 2. Regression plot of the natural logarithm of variant frequency parameters against the concentration of selenium in serum. The natural logarithm (ln) is indicated on the left-hand axes, with the corresponding variant frequency levels on the right-hand axes. (a) Relationship between serum selenium concentration and lnV_f. On single regression analysis, regression coefficient = −0.78 (95% confidence interval −1.66–0.096), $R^2 = 0.031$ ($p = 0.084$, $n = 98$). (b) Relationship between serum selenium concentration and lnV_{fpos}. This parameter relates only to the subjects for which the variant frequency measurement gave a result above the detection limit in the corresponding experiment. On single regression analysis, regression coefficient = −0.96 (95% confidence interval −2.30–0.38), $R^2 = 0.047$ ($p = 0.17$, $n = 42$).

and lnV_{fpos} ($R^2 = 0.060$); this correlation was only significant for lnV_f. In addition, for Antwerp and Peer as well as for Wilrijk and Hoboken considered separately, simple linear regression analysis showed a non-significant positive correlation between *HPRT* mutant frequency and blood lead concentration, in terms of both lnV_f and lnV_{fpos}.

As seen in figure 2, on simple linear regression analysis the concentration of selenium in serum showed a non-significant negative correlation with lnV_f ($R^2 = 0.031$) and lnV_{fpos} ($R^2 = 0.047$).

After the total study population was split into two groups according to blood lead concentration (women with a blood lead level higher than the median versus the others), the group with higher blood lead levels showed, in terms of the crude

data, a higher variant frequency, significantly so for the parameter V_f (geometric mean 7.69×10^{-6} versus 4.64×10^{-6}, $p = 0.019$), whereas the parameter V_{fpos} showed the same trend (geometric mean 9.45×10^{-6} versus 5.21×10^{-6}, $p = 0.108$). After adjustment for age, place of residence, smoking status, level of education, BMI and serum selenium level, the group with higher blood lead levels showed a higher adjusted variant frequency; the difference was significant for the parameter V_f (7.67×10^{-6} versus 4.76×10^{-6}, $p = 0.022$), while the parameter V_{fpos} showed the same trend (9.74×10^{-6} versus 5.06×10^{-6}, $p = 0.077$).

After the total study population was split into two groups according to serum selenium concentration (women with a serum selenium level higher than the median versus the others), the group with higher serum selenium levels showed, in terms of the crude data, a lower variant frequency; the difference was marginally significant for the parameter V_f (geometric mean 4.76×10^{-6} versus 7.23×10^{-6}, $p = 0.051$), and the parameter V_{fpos} showed the same trend (geometric mean 4.99×10^{-6} versus 9.83×10^{-6}, $p = 0.066$). After adjustment for age, place of residence, smoking status, level of education, BMI and blood lead level, the group with higher serum selenium levels showed a lower adjusted variant frequency approaching statistical significance (5.38×10^{-6} versus 6.56×10^{-6}, $p = 0.060$, for V_f; 5.93×10^{-6} versus 8.27×10^{-6}, $p = 0.051$, for V_{fpos}).

Association of HPRT mutant frequencies with other parameters

No consistent trend was observed with regard to the level of education, as both the lowest and the highest level of education were associated with higher variant frequencies.

As *HPRT* V_f and V_{fpos} values were only determined for six and two women, respectively, who were former smokers, a comparison between never-smokers and ex-smokers has only limited value in this study.

Data about passive smoking were available for 53 of the participants. No indication for a contribution of passive smoking to an increase in *HPRT* mutant frequencies was found; on linear regression, variant frequency showed a non-significant negative association with passive smoking both in terms of $\ln V_f$ ($p = 0.15$) and $\ln V_{fpos}$ ($p = 0.55$).

Neither the 'index of consumption of local food' nor the 'frequency of consumption per month of vegetables from own garden' showed a significant correlation with *HPRT* variant frequency in Antwerp or in Peer. This was also the case in terms of linear regression analysis with $\ln V_f$ and $\ln V_{fpos}$. The corresponding standardized regression coefficients were negative or positive but were always very low in absolute value, with p values between 0.68 and 0.90 for $\ln V_f$ and between 0.25 and 0.70 for $\ln V_{fpos}$. After correction for place of residence, smoking status, level of education, age, BMI and serum selenium level, women consuming two or more types of local food did not show a significantly higher variant frequency than women who did not consume local food in terms of V_f (adjusted $V_f = 4.90 \times 10^{-6}$ for the former versus 4.79×10^{-6} for the latter, $p = 0.84$) or V_{fpos} (adjusted $V_{fpos} = 4.75 \times 10^{-6}$ for the former versus 4.92×10^{-6} for the latter, $p = 0.36$).

Discussion and conclusion

As significant differences in *HPRT* variant frequency were observed in association with place of residence and after correction for personal and lifestyle factors, our data add to the growing body of evidence indicating that environmental exposures can increase mutation rates (Perera *et al.* 1992, 2002, Manchester *et al.* 1995, Fu *et al.* 1999, Michalska *et al.* 1999).

In addition, in women without occupational exposure and with blood lead levels within 'normal' limits, higher blood lead levels were, to some extent, associated with higher *HPRT* mutant frequencies. This trend towards higher mutant frequencies (correlation coefficient = 0.228 in linear regression, $R^2 = 0.052$) was statistically significant only for the parameter V_f, which could be calculated for all subjects in the study and which also included subjects for whom the actual variant frequency was below the detection limit (in whom V_f was assumed to be equal to the detection limit in the corresponding measurement). However, the same trend (correlation coefficient = 0.245 in linear regression, $R^2 = 0.060$) was present for the parameter V_{fpos}, which only included variant frequency values above the detection limit, but statistical significance was not reached due to lower numbers. To our knowledge, an association between blood lead levels and mutant frequency in peripheral blood lymphocytes in humans has not been reported before. There are, however, quite a few indications in the literature pointing towards a possible causal relationship between lead exposure and genotoxic events. Several authors have reported the mutagenic effects of lead in mammalian cells *in vitro* (Zelikoff *et al.* 1988, Hartwig *et al.* 1990, Roy and Rossman 1992, Yang *et al.* 1996). Inhaled lead was found to be genotoxic in CD-1 mice (Valverde *et al.* 2002), and in humans a weak but significant correlation was found between the concentration of lead in seminal plasma and 8-hydroxy-2′-deoxyguanosine in sperm DNA (Xu *et al.* 2003). In our previous study on urban pigeons exposed to air pollution, an association was observed between oxidative DNA damage and lead levels in liver (Schilderman *et al.* 1997). In contrast, Merzenich *et al.* (2001) could not find a relationship between blood lead levels and the comet assay response in inhabitants of Bremen. However, exposure to lead was associated with genetic damage in peripheral blood lymphocytes of workers as assessed through increased frequency of sister chromatid exchanges (Rajah and Ahuja 1995, Dóonmez *et al.* 1998, Duydu *et al.* 2001), as measured by the comet assay (Restrepo *et al.* 2000), or through an increase in micronuclei (Vaglenov *et al.* 2001). There is ample experimental evidence that inorganic lead is carcinogenic (Silbergeld *et al.* 2000) and it is classified by International Agency for Research on Cancer (IARC) as group 2B (i.e. possibly carcinogenic to humans). Jemal *et al.* (2002) found a non-significant trend towards higher total cancer mortality with increasing blood lead levels in the normal range. It is speculated that lead may exert an indirect carcinogenic action by lowering intracellular glutathione levels, as well as inhibiting DNA repair. Although Asmuss *et al.* (2000) reported that lead did not inhibit the oxidative DNA damage-repairing formamidopyrimidine DNA glycosylase *in vitro*, alternatively lead may replace zinc in numerous zinc-binding proteins, such as superoxide dismutase and p53. This may result in impaired antioxidative defence as well as reduced DNA repair.

The (non-significant) trend towards lower *HPRT* mutant frequencies in women with higher serum selenium levels that we observed might be in concordance with data indicating a protective effect of a higher selenium intake against genetic damage in humans (El Bayoumy 2001).

In this study the impact of specific dietary habits on *HPRT* mutant frequency did not appear to be very pronounced. In particular, we were not able to observe a clear effect of the consumption of locally produced food on *HPRT* mutant frequencies. Alcohol intake was not associated with higher mutant frequency values, in accordance with the findings of Cole and Green (1995). We could not confirm the positive correlation between vitamin A and *HPRT* mutant frequency, or the negative correlation between total fat and *HPRT* mutant frequency found by Branda and Albertini (1995). We only had data on recent dietary habits and these data were of limited quality; this may have contributed to the fact that few differences were observed. In this study we did not observe a significant increase of *HPRT* mutant frequencies with age, in accordance with Curry *et al.* (1999), who found that after the age of 53 years the *HPRT* mutant frequency is largely stable.

The rural population, originally selected as a control population, showed higher levels for some exposure biomarkers (table 4 and unpublished results) and also had higher *HPRT* mutant frequencies than the population residing in an industrial urban area. This indicates that pollution is widespread in Flanders, and suggests that in developed Western nations exposure to pollutants may be quite homogeneous. Our data also suggest that negative confounding may well be an often overlooked problem in relation to the study of the biological and health effects of pollution, as rural populations used as control might, as is the case here, experience more intense exposures and biological effects than presumably 'exposed' persons.

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