CYP1A1 Messenger RNA Levels in Placental Tissue as a Biomarker of Environmental Exposure[1]

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Abstract

The human *CYP1A1* gene codes for an inducible enzyme system involved in biotransformation of certain xenobiotics, including polycyclic aromatic hydrocarbons; some of the metabolites are carcinogenic and mutagenic. Effects of environmental exposures (smoking, air pollution, and diet) on *CYP1A1* gene induction in placental tissue and the modulation of induction by the *CYP1A1 Msp*I RFLP were evaluated in two groups from Poland: 70 mother-child pairs from Krakow, a city with elevated air pollution; and 90 pairs from Limanowa, a less polluted area. Compared to placentas from nonsmoking women, *CYP1A1* mRNA levels were significantly increased in placentas from current smokers ($P < 0.001$). Ex-smokers also had significantly higher placental mRNA levels, including women who quit smoking prior to pregnancy ($P < 0.01$). A marginal increase in *CYP1A1* mRNA with environmental tobacco smoke exposure was evident. Within Krakow, there was an increase in *CYP1A1* mRNA with ambient pollution at the place of residence for each woman, which was significant among women who were not employed away from the home ($P < 0.05$ controlling for smoking status, diet, and use of coal for heating). Significant increases in mRNA were associated with dietary consumption of smoked meat, cheese, and fish ($P < 0.01$). The *CYP1A1 Msp*I RFLP was not a significant determinant of *CYP1A1* mRNA levels after controlling for smoking and other variables. Human placenta provides a readily available and responsive system that can serve as a model for evaluating environmental and genetic determinants of *CYP1A1* induction.

Introduction

Differences in the metabolic activation and detoxification pathways for carcinogens are likely to be a major source of interindividual variation in cancer risk. Therefore, improved understanding of the nature and significance of human variation in susceptibility and response has been designated a priority in cancer prevention (1, 2). Cytochrome *CYP1A1* is involved in the biotransformation of certain xenobiotics including PAHs,[3] to phenolic products and epoxides, some of which are toxic, mutagenic, and carcinogenic (3). AHH and EROD are measures of *CYP1A1* gene induction (4–6). Both have been shown to be highly inducible enzyme systems in human placenta and other tissues following exposures to PAHs (5, 7). Genetic differences in induction may mediate susceptibility to PAH-induced carcinogenesis (8–10). Human placenta is a readily available, *CYP1A1*-inducible system that can serve as a model for investigating environmental and genetic determinants of *CYP1A1* induction (7, 11). Induction of placental AHH and EROD activity as a result of maternal smoking has been well documented (5, 12–14). PAHs in cigarette smoke are presumed to be responsible for these enhanced enzyme activities (12). Previous studies suggest an association between AHH and EROD activity in placental tissue and ETS exposure (12, 15). One study found an association between placental AHH activity and severe ambient air pollution (16).

Several studies report an increase in EROD activity in lymphocytes in those that are heterozygote for the *CYP1A1 Msp*I RFLP or those with the mutation in exon 7 of the gene (17, 18). The exon 7 mutation, which has been associated with the *CYP1A1 Msp*I RFLP, results in replacement of isoleucine by valine near the heme-binding region of *CYP1A1* (19). *In vitro* results indicate that the valine-type *CYP1A1* protein may have higher AHH activity than the isoleucine-type or "normal" protein (20). However, in a previous study, no association was seen between the polymorphism and *CYP1A1* mRNA inducibility in human lymphocytes (21).

Direct measurement of *CYP1A1* gene inducibility may be more reproducible than AHH activity (4). The current study extends prior research on the applicability of *CYP1A1* gene induction as a biologically relevant dosimeter for en-

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbons; AHH, aryl hydrocarbon hydroxylase; EROD, 7-ethoxyresorufin *O*-deethylase; ETS, environmental tobacco smoke; BP, benzo(*a*)pyrene; PM_{10}, ambient particulate <10 μm.

vironmental exposures to PAHs (22). It augments previous investigations of placental AHH and EROD activity.

We have evaluated the association between placental *CYP1A1* mRNA levels and ambient air pollution, cigarette smoking (both active and passive), and dietary consumption of broiled, fried, and smoked meats, and smoked cheese and fish. The study also examines the relationship between gene inducibility and the *CYP1A1 Msp*I RFLP. A better understanding of individual environmental and genetic determinants of an enzyme system which bioactivates xenobiotics at the interface between maternal and fetal circulation may be relevant to assessing the risk of fetal exposures to PAHs and other transplacental carcinogens.

Materials and Methods

Study Subjects and Data Collection. During January to March, 1992, 70 mother/newborn pairs were enrolled in the study from the University Hospital in Krakow, Poland, an industrial city with severe ambient air pollution. As controls, 90 mother/newborn pairs were enrolled from the Regional Hospital in Limanowa, Poland, a small town 70 km southeast of Krakow in a rural agricultural district. On the basis of ambient monitoring data from the Division of National Sanitary Inspection of Poland, ambient levels of respirable particulates in Limanowa are 5–6-fold lower than in Krakow. Enrollment alternated on a biweekly basis between Krakow and Limanowa to control for monthly variations in ambient pollutant levels and seasonal variations in AHH enzyme activity. Enrollment was restricted to women who had resided in Krakow or Limanowa for at least 1 year and was limited to vaginal deliveries.

Immediately after delivery, a sample of umbilical cord blood (20–60 ml) was collected. The decidua was removed from the placenta and a 15-g sample of villus (fetal) placental tissue was collected from the four quadrants and the center of the placenta and placed immediately into liquid nitrogen. Within 12 h of delivery, the cord blood was centrifuged and plasma samples were collected and stored in liquid nitrogen. A sample of maternal venous blood (20–35 ml) was collected within 2 days postpartum and processed, as were the umbilical cord blood samples. Placental tissue and plasma samples were shipped from Poland to Columbia University on dry ice and stored at −70°C.

A detailed and validated questionnaire that was administered to the mother 1–2 days postpartum included information on smoking, residential and employment histories, use of coal stoves for residential heating, and non-air pollution-related exposures. While a dietary survey was not administered, subjects were asked to estimate the average number of servings consumed per week during pregnancy of specific foods. These included broiled and fried meats and smoked meats, cheese, and fish as potential dietary sources of PAHs. All interviews were conducted by two trained interviewers from the Department of Epidemiology and Preventive Medicine, University Medical School, Krakow. Interviews were coded, and coded data were sent to Columbia University.

Assessment of smoking status was based on questionnaire data. Current smokers had smoked ≥1 cigarette/day for ≥6 months during their lifetimes and were smoking up to delivery. Ex-smokers had smoked ≥1 cigarette/day for ≥6 months during their lifetimes but had quit ≥1 month prior to delivery. Ex-smokers were further dichotomized into those who quit prior to and during pregnancy. Nonsmokers had never smoked ≥1 cigarette/day for ≥6 months.

Plasma cotinine levels were used to verify questionnaire data on smoking status. Plasma cotinine provides a marker for recent cigarette smoke exposure (half-life, approximately 18 h in smokers to 48 h in nonsmokers) (23). While plasma cotinine levels associated with the heavy ETS exposure encountered in Poland have not been well characterized, levels of 5–25 ng/ml have been documented with passive exposure in other studies, and levels >25 ng/ml have been generally considered incompatible with ETS exposure and indicative of active smoking (24, 25). In the current study, ex-smokers and nonsmokers with maternal plasma cotinine levels >25 ng/ml were identified, and analyses were performed both including (with smoking status based on questionnaire data) and excluding these subjects. Reclassification of these individuals as current smokers based on plasma cotinine was rejected because plasma cotinine levels associated with ETS exposure in Poland have not been adequately characterized to allow definite determination of the cutoff.

Daily ambient monitoring data were collected from Krakow for 1990–1992 from the Division of National Sanitary Inspection (15 monitoring stations) and the United States Environmental Protection Agency (5 monitoring stations). Exposure of each Krakow subject was then estimated by taking the average of PM_{10} measurements (in $\mu g/m^3$) reported at the monitoring station closest to her residence for each of the past 2 years and the month prior to her delivery date. Ambient particulate data were available for 69 of 70 subjects from Krakow. Because there was only one ambient monitoring station in Limanowa, individual ambient exposures could not be estimated for Limanowa subjects.

***CYP1A1* Gene Expression Assay.** Frozen placental samples were thawed, and each sample was homogenized directly in guanidinium thiocyanate. Total RNA was isolated as described previously (21). Slot-blot analyses were performed on glyoxal-denatured RNA samples, with filter hybridization to nick-translated, ^{32}P-labeled cDNA probes, by previously established procedures (22). The human *CYP1A1* probe (phP1-450-3′) and the human β-actin probe (phFβA-1) were obtained from the American Type Culture Collection (Bethesda, MD). Following final filter washes of 0.4× SSC (1× SSC = 3 M NaCl-0.3 M sodium citrate) and 0.1% SDS, *CYP1A1* and actin mRNA were visualized by film autoradiography, and signal strengths were quantified by the measurement of optical densities with a scanning laser densitometer (LKB Ultroscan). All *CYP1A1* gene expression results were normalized to actin expression, which served as an internal standard to ensure equal loading of RNA onto filters.

***Msp*I RFLP.** High molecular weight genomic DNA from placental villus (fetal) samples was isolated/extracted by phenol-chloroform as described previously (21). Following extraction, 10 μg of DNA samples were digested with 20 units of *Msp*I (Boehringer-Mannheim, Indianapolis, IN) for 16 h at 37°C following the manufacturer's instructions. The resultant DNA fragments were electrophoretically separated on 1.2% agarose slab gels and transferred onto nylon membrane filters (Nytran; Schleicher and Schuell, Keen, NH) by methods described previously (26). Hybridization of filter-bound DNA to radiolabeled cDNA probes was performed as described above, and following a high-strin-

gency final wash of 0.1× SSC and 0.1% SDS, visualization of hybridized DNA fragments was accomplished by film autoradiography. Mobility of all DNA fragments was measured, and molecular size (in kilobases) was determined by comparison to known DNA standards.

The PCR-RFLP approach was also used to determine *CYP1A1 Msp*I genotype using DNA from umbilical cord blood samples. For these, 100 ng of genomic DNA was added to a PCR containing oligonucleotide primers that flack the *Msp*I site in the *CYP1A1* gene (19). PCR products were digested with *Msp*I and resolved on a 3% Nusieve/agarose gel (FMC Corp. BioProducts, Rockland, ME).

Plasma Cotinine. The method involved liquid/liquid extraction of plasma, followed by gas chromatographic separation using a 30-m 0.25 μ DB WAX megabore column and a nitrogen-phosphorus detector operated in the nitrogen mode. An internal standard, *N*-methyl cotinine, was added to the plasma before extraction. Five-point standard curves were generated for each analytical run, and low and high quality control samples were processed each day. The method requires cold trapping injection followed by temperature programming to achieve optimal separation resulting in an analysis time of 18 min/sample. To facilitate productivity, an autosampler and online automatic data reduction were used so that samples could be processed during the evening or overnight, as needed. The standard curves are linear throughout the expected range with intra- and inter-relative SD percentages of 3.8 and 4.5, respectively.

Statistical Analysis. *CYP1A1* mRNA values in placental tissue were log transformed to stabilize the variance and to obtain a more symmetrical distribution. For placentas with nondetectable levels, a mRNA level of one-half the detection limit (0.01) was assigned prior to log transformation. Means and SEs are presented as untransformed values for ease of interpretation. Two measures of ambient pollution were tested: residence in the higher (Krakow) *versus* lower (Limanowa) pollution areas and ambient particulate (PM_{10}) levels (Krakow). The associations between the log-transformed mRNA level and ambient pollution, smoking status, diet, and genotype (*CYP1A1 Msp*I RFLP) were examined initially by Student's *t* test. A literature search was conducted to identify potential confounders. χ^2 and *t* test were used to test the difference between Krakow and Limanowa subjects for potential confounders. The associations between *CYP1A1* mRNA and environmental exposures and genotype were tested by multiple linear regression and analysis of covariance (ANCOVA). The final model included place of residence (Krakow *versus* Limanowa) or ambient pollution groups based on particulate levels (Krakow only), cigarette smoking status, average number of servings per week (during pregnancy) of smoked meat, cheese and fish (diet), and whether subjects used coal stoves for residential heating (coal use). For the association between *CYP1A1* mRNA and genotype, dietary information was adjusted by assigning the mean number of servings per week of smoked foods (calculated for all subjects) to the two subjects for whom this dietary information was missing. This was necessary since one of two subjects was homozygous for the *CYP1A1 Msp*I RFLP.

Results

Demographic data are presented in Table 1. Placental *CYP1A1* mRNA levels were available from 155 mother/child pairs including 67 pairs from Krakow and 88 pairs from Limanowa. Data are presented here for the 155 mother/child pairs. Mothers in Krakow were older than mothers in Limanowa. Of the 155 mothers, 15 were current smokers, 36 were ex-smokers, and 104 were nonsmokers. Current smokers averaged 9.1 ± 8 cigarettes/day during pregnancy with maternal plasma cotinine levels consistent with light smoking (mean, 37 ± 39 ng/ml). Ex-smokers smoked an average of 8.9 ± 5.9 cigarettes/day before pregnancy. Twenty ex-smokers reported smoking during the first trimester of pregnancy, with 5 smoking regularly (10 ± 7.7 cigarettes/day), but only 1 woman continued to smoke regularly during the second trimester (2 cigarettes/day). None of the ex-smokers reported smoking regularly during the final trimester.

Table 1 Demographics

	Krakow	Limanowa	Total
Mother/child pairs	67	88	155
Mother's age (yr)	27.6 ± 5.4	25.3 ± 4.1[a]	26.3 ± 4.8
Current smokers	11[b]	4	15
Ex-smokers	19[b]	17	36
Nonsmokers	37	67	104
ETS exposure	42	61	103
Heating by coal stove	15	43[c]	58
*CYP1A1 Msp*I (−/−)	50	73	123
*CYP1A1 Msp*I (+/−,+/+)	17	15	32
Smoked foods eaten ≥1/week	33	38	71
Broiled/fried meat eaten ≥1/week	52	71	123

[a] Average age of mothers in Krakow was significantly greater than average age in Limanowa ($P < 0.01$).
[b] Significantly more of the cohorts from Krakow (45%) than from Limanowa (24%) had a history of smoking, *i.e.*, current, or ex-smokers ($P < 0.01$).
[c] Significantly more of the subjects from Limanowa (49%) than Krakow (22%) used coal stoves for residential heating ($P < 0.01$).

Five of the ex-smokers and three of the nonsmokers had maternal plasma cotinine levels >25 ng/ml. Analyses were performed both including and excluding these subjects. Except as reported, the associations between *CYP1A1* mRNA and cigarette smoke exposure (active and passive) were not changed when the eight subjects were excluded. Results are presented here for the total cohort with smoking status based on the questionnaire data unless stated otherwise.

In addition to active cigarette smoke exposure, 66% (103 of 155) of the cohort reported exposure to ETS during the last 2 years (with an average of 14.3 ± 14.2 cigarettes/day smoked in their presence), and 54% (84 of 155) continued to be exposed to ETS during pregnancy (with an average of 12.1 ± 11.9 cigarettes/day smoked in their presence).

For the *CYP1A1 Msp*I RFLP, 123 (79%) infants were homozygous with the normal 2.3-kilobase DNA fragment (*CYP1A1 Msp*I−/−), 29 (19%) were heterozygous with both the 2.3-kilobase and the variant 1.9-kilobase fragment (*CYP1A1 Msp*I−/+), and 3 (2%) were homozygous-variant with only the 1.9-kilobase fragment (*CYP1A1 Msp*I+/+).

Forty-six % (71 of 155) of the subjects consumed smoked meat, cheese, and fish on average ≥1/week during pregnancy, while 79% (123 of 155) consumed broiled or fried meats ≥1/week.

Table 2 *CYP1A1* mRNA levels by cigarette smoke exposure (active and passive) and *CYP1A1 MspI* RFLP

	Active smoking	ETS+	ETS−	*CYP1A1 MspI*−/−	*CYP1A1 MspI*+/−	*CYP1A1 MspI*+/+
Total cohort	0.46 ± 0.09 (155)[a]	0.57 ± 0.13 (103)	0.22 ± 0.03 (52)	0.39 ± 0.08 (123)	0.66 ± 0.31 (29)	1.17 ± 0.71 (3)[b]
Current smoker (CS)[c]	1.49 ± 0.45 (15)[d]	1.69 ± 0.55 (12)	0.71 ± 0.17 (03)	1.44 ± 0.51 (11)	2.09 ± 1.43 (3)	0.27 ± 0 (1)
<10 cigs./day	0.67 ± 0.19 (07)					
≥10 cigs./day	2.46 ± 0.83 (07)[e]					
Ex-smoker (EX)	0.77 ± 0.28 (36)[f]	0.91 ± 0.36 (28)	0.27 ± 0.06 (08)	0.55 ± 0.23 (29)	1.52 ± 1.28 (6)	2.57 ± 0 (1)
Last cig > 9 months	0.82 ± 0.41 (16)[g]					
Last cig ≤ 9 months	0.73 ± 0.40 (20)[g]					
Nonsmoker (NS)	0.20 ± 0.04 (104)	0.21 ± 0.06 (63)	0.18 ± 0.03 (41)	0.20 ± 0.04 (83)	0.18 ± 0.03 (20)	0.66 ± 0 (1)

[a] Results show mean ± SE (*n*).
[b] $P < 0.05$ *CYP1A1 MspI*+/+ compared to *CYP1A1 MspI*−/− (Student's *t* test).
[c] CS, current smoker; EX, ex-smoker; cigs., cigarettes; NS, nonsmoker.
[d] $P < 0.001$ CS compared to NS; $P < 0.01$ CS compared to EX (Student's *t* test).
[e] $P = 0.09$ CS ≥10 compared to CS <10 cigs. (Student's *t* test).
[f] $P < 0.01$ EX compared to NS (Student's *t* test).
[g] $P < 0.05$ EX > 9 months and ≤ 9 months compared to NS (Student's *t* test).

Table 3 Regression model

Dependent variable: Log-transformed placental *CYP1A1* mRNA levels.
Independent variables: Smoking status, place of residence, coal use, diet.

	β	*P* value
Current smokers *vs.* nonsmokers	2.2	<0.001
Ex-smokers *vs.* nonsmokers	1.0	<0.001
Servings/week of smoked foods	0.2	<0.01
Coal stoves used for heating	0.3	0.26
Place of residence	−0.4	0.14

Table 4 Correlation of plasma cotinine (maternal and infant cord) and days since last cigarette with placental *CYP1A1* mRNA (controlling for place of residence, coal use, and diet)

	Partial *r*	*P* value
Full cohort (*n* = 155)		
Maternal plasma cotinine	0.46	<0.001
Cord plasma cotinine	0.47	<0.001
Days since last cig.[a]	NS	NS
Current smokers (*n* = 15)		
Maternal plasma cotinine	0.66	0.04
Cord plasma cotinine	0.80	<0.01
Days since last cig.	−0.60	0.07

[a] cig., cigarette; NS, not significant.

Significantly more of the subjects from Krakow than Limanowa reported a history of smoking, and significantly more of the subjects from Limanowa than Krakow reported use of coal stoves for residential heating. There was no significant difference between Krakow and Limanowa in subjects reporting either ETS exposure or dietary consumption of broiled, fried, or smoked foods, nor in distribution of the *CYP1A1 MspI* RFLP.

CYP1A1 mRNA levels stratified by cigarette smoke exposure (active and passive) and by the *CYP1A1 MspI* RFLP are presented in Table 2. The major determinant of *CYP1A1* mRNA levels in placental tissue was active smoking. Levels among current smokers were significantly increased compared to nonsmokers both in the univariate analysis ($P < 0.001$; Student's *t* test; Table 2) and after adjusting for place of residence, diet, and coal use ($P < 0.001$; Table 3). In separate analyses, a highly significant correlation was seen between placental *CYP1A1* mRNA and both maternal and infant cord blood plasma cotinine levels in the total cohort ($P < 0.001$) and among current smokers only ($P < 0.05$; Table 4). Among current smokers, placental *CYP1A1* mRNA levels were inversely correlated with the number of days that had elapsed since the mother smoked her last cigarette (range, 0–14 days; partial $r = -0.60$; $P = 0.07$; Table 4), indicating that *CYP1A1* declines fairly rapidly following smoking cessation. No significant association between mRNA levels and number of cigarettes smoked by the mother per day was seen in the regression analysis. However, current smokers of ≥10 cigarettes/day had higher *CYP1A1* mRNA levels than current smokers of <10 cigarettes/day (2.46 *versus* 0.67; $P = 0.09$; Table 2).

Placental tissue from ex-smokers also had significantly increased *CYP1A1* mRNA levels compared to that from nonsmokers both in the univariate analysis (Table 2) and after controlling for place of residence, coal use, and diet ($P < 0.001$; Table 3). *CYP1A1* mRNA levels were significantly increased in both long-term ex-smokers who quit prior to pregnancy and more recent ex-smokers who quit during pregnancy ($P < 0.01$ by ANCOVA controlling for place of residence, diet, and use of coal). Ex-smokers who quit prior to pregnancy still had marginally increased placental *CYP1A1* mRNA levels after the eight ex- and nonsmokers with plasma cotinine >25 ng/ml had been removed from the analysis ($P = 0.09$ by ANCOVA controlling for the same variables).

As seen from Table 2, placental *CYP1A1* mRNA levels were higher in subjects reporting ETS exposure both in the total cohort and after stratification by smoking status. However, the differences were not significant both before and after controlling for the potential confounders.

Results of placental *CYP1A1* inducibility by ambient pollution and coal use for residential heating are presented in Table 5 and Fig. 1. Overall, there was no difference in mean *CYP1A1* mRNA levels between Krakow and Limanowa subjects. Placental *CYP1A1* mRNA levels were 2-fold higher among Limanowa subjects reporting use of coal for heating compared to noncoal users, a difference that was of borderline significance ($P = 0.08$) by Student's *t* test (Table 5). Among noncoal users, levels were 1.5-fold higher in subjects from Krakow (the higher pollution area)

Table 5 Placental *CYP1A1* mRNA place of residence and use of coal for residential heating[a]

	Total cohort	No coal used	Coal used
All	0.46 ± 0.09 (155)	0.36 ± 0.10 (95)	0.62 ± 0.17 (58)
Krakow	0.47 ± 0.14 (67)	0.43 ± 0.16 (51)	0.61 ± 0.32 (15)
Limanowa	0.45 ± 0.11 (88)	0.29 ± 0.10 (45)	0.62 ± 0.20 (43)[b]

[a] Results show mean ± SE (*n*).
[b] $P = 0.08$; Limanowa subjects using coal stoves compared to Limanowa subjects not using coal stoves for residential heating (Student's *t* test).

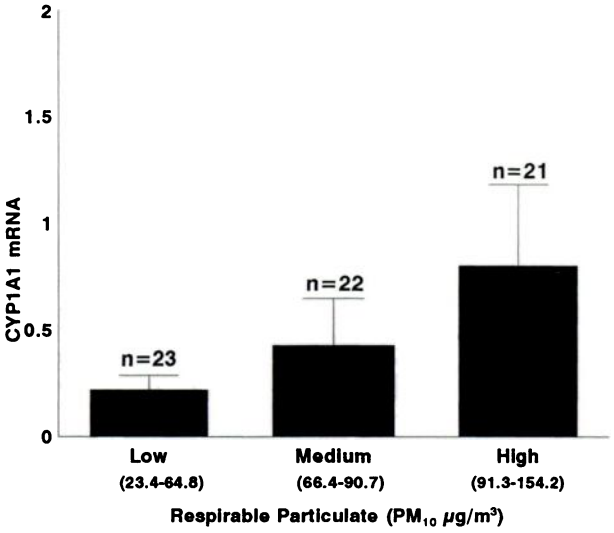

Fig. 1. Placental *CYP1A1* mRNA by level of PM_{10} pollution the month prior to delivery for 66 mother/newborn pairs from Krakow, Poland. *CYP1A1* mRNA levels were higher in placentas from women in the high compared to low pollution group ($P = 0.06$; Student's *t* test). *Columns,* mean; *bars,* SE.

compared to Limanowa (the lower pollution area; Table 5). Estimated ambient concentrations of PM_{10} for Krakow subjects at their place of residence during the month prior to delivery averaged 80.5 μg/m³ (range, 23.4–154.2 μg/m³). When the Krakow cohort was trichotomized into low, medium, and high pollution groups based on PM_{10}, a dose-related increase in *CYP1A1* mRNA with ambient exposures was suggested (Fig. 1). Placental *CYP1A1* mRNA levels were 3.7-fold higher in placentas from women residing in the high compared to low pollution group ($P = 0.06$; Student's *t* test). Comparing Krakow and Limanowa residents who did not use coal for residential heating, placental *CYP1A1* mRNA levels were 3.4-fold higher among Krakow subjects ($n = 15$) residing in the high pollution group than among Limanowa subjects ($n = 45$; $P = 0.05$; Student's *t* test). In Krakow residents, the association between placental *CYP1A1* mRNA and exposure to ambient particulates was most evident among cohort members who were not employed away from the home. This is biologically plausible since unemployed women are likely to spend most of their time at their place of residence. Among the unemployed, a significant increase in placental *CYP1A1* mRNA levels was seen in univariate analysis between subjects residing in the low pollution group (0.10 ± 0.03) compared to subjects residing in both the middle (0.25 ± 0.04; $P = 0.01$) and high (0.72 ± 0.34; $P = 0.04$) pollution groups

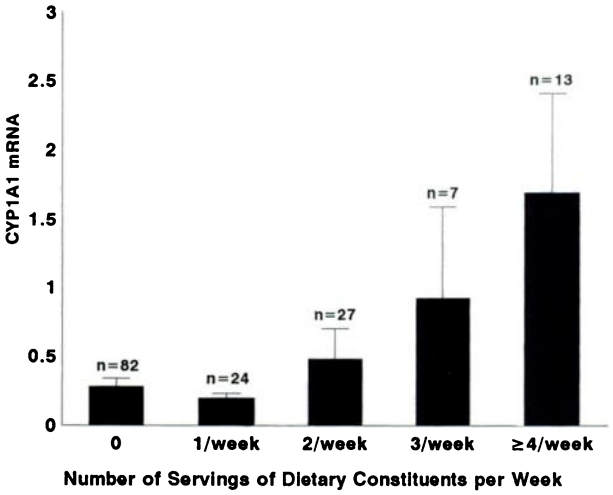

Fig. 2. Placental *CYP1A1* mRNA levels by average number of servings of smoked foods (meat, cheese, and fish) consumed per week during pregnancy. Women consuming smoked foods ≥4 times/week had significantly increased placental mRNA levels compared to never consumers ($P < 0.01$; Student's *t* test). *Columns,* mean; *bars,* SE.

(Student's *t* test). By ANCOVA, controlling for smoking status and diet, neither the association of *CYP1A1* mRNA with place of residence (Krakow *versus* Limanowa) nor use of coal stoves for residential heating was statistically significant. However, among unemployed subjects within Krakow, the difference in *CYP1A1* mRNA levels between ambient pollution groups remained significant after controlling for smoking status, diet, and coal use ($P = 0.04$; ANCOVA).

Dietary consumption of smoked meat, cheese, and fish was a significant determinant of placental *CYP1A1* mRNA levels. As seen from Fig. 2, a dose response in mRNA with increasing number of servings per week of these dietary constituents was evident. Compared to never consumers, mRNA level were significantly higher in those consuming smoked meat, cheese, and fish ≥4/week ($P < 0.01$; Student's *t* test). After controlling for smoking status, place of residence, and coal use, the correlation between *CYP1A1* mRNA levels and self-reported number of servings of these smoked foods per week during pregnancy was highly significant ($P < 0.01$; Table 3). No significant association was seen between mRNA levels and consumption of broiled or fried meats, nor was any significant association seen between mRNA and the other dietary constituents (coffee, tea, and alcohol) assessed.

Determination of the *CYP1A1 Msp*I RFLP was completed on 137 of 155 umbilical cord DNA samples and on 148 of 155 placenta samples. Both provide data on the genotype of the infant because the placental (villus) tissue collected was fetal tissue. Those subjects for whom the RFLP was determined by both methods, the concordance was 100%. As seen from Table 2, compared to subjects with the homozygous normal genotype (*CYP1A1 Msp*I–/–), *CYP1A1* mRNA levels were 1.7-fold higher in subjects with the heterozygous variant genotype (*CYP1A1 Msp*I+/–) and 3-fold higher in subjects with the homozygous variant genotype (*CYP1A1 Msp*I+/+; $P = 0.05$; Student's *t* test). When stratified by smoking, *CYP1A1* mRNA levels were generally higher in heterozygotes or homozygotes compared to those with the normal genotype (Table 2) although sample size

was small and none of the differences were statistically significant. Further, the polymorphism was not a significant determinant of *CYP1A1* mRNA levels in the regression analysis controlling for smoking status, place of residence, coal use, and diet (adjusted). Nor was the correlation in the regression analysis between plasma cotinine and *CYP1A1* mRNA levels stronger in those heterozygote and homozygote for the RFLP compared to those with the normal genotype. (For maternal cotinine, the partial *r* between cotinine and mRNA levels was 0.47 for heterozygotes and homozygotes combined compared to 0.46 for normals, while for infant cotinine the comparison was 0.43 for heterozygotes and homozygotes compared to 0.47 for normals.) As anticipated, therefore, no significant interaction between the effects of the *CYP1A1 Msp*I genotype (heterozygotes and homozygotes combined) and either smoking status or plasma cotinine (dichotomized into $\leq$ or $>$ the median value) on *CYP1A1* mRNA levels was seen in the regression analysis.

Discussion

We found a highly significant association between active cigarette smoking status and placental *CYP1A1* mRNA levels. Plasma cotinine levels were also strongly associated with the mRNA levels in the total cohort and among current smokers. Among the latter, plasma cotinine predicted placental *CYP1A1* mRNA better than did questionnaire-derived cigarettes smoked per day. In a previous study no effect of smoking was seen on *CYP1A1* mRNA level in peripheral lymphocytes (22). The present results suggest that placenta is a more useful tissue than are lymphocytes for studying the effect of exposure on gene expression.

Our results indicate that placental *CYP1A1* mRNA levels decline rather rapidly following smoking cessation. A rapid decline in *CYP1A1* mRNA has also been seen in normal lung tissue, with a time-dependent decrease occurring within 2 weeks (27).

However, while *CYP1A1* mRNA levels decline following smoking cessation, they do not appear to return to nonsmoker levels. In fact, placental *CYP1A1* mRNA levels were still significantly increased in long-term ex-smokers who quit prior to pregnancy as well as in more recent quitters. One possible explanation for this finding is that long-term ex-smokers have misreported their smoking status and/or the elapsed time since last cigarette. After the eight ex-smokers and non-smokers with plasma cotinine levels >25 ng/ml were removed from the analysis, mRNA levels were still increased, although the difference was of borderline significance. An alternative explanation is that PAHs from cigarette smoke are stored in body lipids during periods of active smoking and are released into the blood stream during pregnancy, causing induction of placental *CYP1A1*. Experimental data indicate that mammary and other fat tissues can be significant storage depots for PAHs (28, 29). Consistent with our findings, several prior studies on placental AHH report that activity is significantly higher in ex-smokers compared to nonsmokers (15, 16). However, these prior cohorts have been restricted principally to women who quit smoking during pregnancy; the association between long-term smoking cessation and enzyme activity could not be evaluated.

A modest nonsignificant increase in placental *CYP1A1* mRNA was seen with ETS exposure. Prior studies have suggested an association between current ETS exposure and placental AHH and EROD activity (12, 15). Additional studies of larger sample size are needed to explore this association more fully.

CYP1A1 mRNA levels were marginally higher in subjects reporting use of coal for residential heating and, within Krakow, a dose response with ambient particulate levels was suggested. The difference in *CYP1A1* mRNA levels between pollution groups in Krakow was significant for those women not employed away from the home during pregnancy. A principal source of ambient pollution within Krakow is the large number of coal-burning stoves, with the heaviest pollution found in the older, central section of the city. A prior case-control study from Krakow found a significant association between lung cancer and residence in this central, high-polluted area (30). A strong association between placental AHH activity and ambient air pollution was seen in one study from Turkey (16). *In vitro* experiments also indicate that AHH activity can be induced by extracts of particulates collected from urban air (31). Ambient PAH levels were not available for Krakow. However, ambient BP levels in adjacent Silesia, Poland (which is comparable to Krakow in terms of coal use) range from 0.02–0.06% of ambient particulate levels (32). If the ratio is similar for Krakow, BP levels in the highest pollution stratum could average between 18 and 93 ng/m^3, which is close to BP exposures seen in our previous research on foundry workers (33). In contrast, annual average airborne BP levels in the United States generally range from 2 to 3 ng/m^3 with exceptions in heavy coal-burning states such as Illinois, Ohio, and Pennsylvania (34).

To our knowledge, this is the first study to show an association between either placental *CYP1A1* gene induction or enzyme activity and dietary consumption of smoked meats, cheese, and fish. These results require confirmation. However, the association is biologically plausible. PAHs are common contaminants of cooked foods including those that have been broiled and smoked (35). A prior study found no association between placental AHH activity and consumption of charcoal-broiled beef (11). However, a significant association between PAH-DNA adducts in peripheral leucocytes and consumption of charcoal-broiled food within the previous week was seen in another study (36).

Placental *CYP1A1* levels were increased in those heterozygous and homozygous for the *CYP1A1 Msp*I RFLP compared to those with the normal genotype. However, the association was not significant once smoking and other variables had been controlled. A previous study found no association between the polymorphism and *CYP1A1* mRNA inducibility in human lymphocytes (21, 37).

In conclusion, measurement of placental *CYP1A1* mRNA appears to provide an informative marker of environmental PAH exposure, including from cigarette smoke, ambient air, and diet. It should be noted that exposure estimates for PM_{10} are based on ambient air-monitoring data and are not of the same precision as other variables analyzed, particularly cigarette smoke exposure, for which exposure estimates were based both on detailed questionnaire data and plasma cotinine levels. Nor should the association between exposure and placental *CYP1A1* gene induction be assumed to be straightforward because interindividual variability is due to genetic determinants as well as exposures. Environmental and genetic determinants of an inducible enzyme system that bioactivates xenobiotic at the interface between maternal and fetal circulation may be an important factor in transplacental DNA damage and carci-

nogenesis. Future studies will evaluate the association between placental *CYP1A1* gene induction and PAH-DNA adduct formation in placental tissue and infant cord blood leucocytes.

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