The Use of 4-Aminobiphenyl Hemoglobin Adducts and Aromatic DNA Adducts in Lymphocytes of Smokers as Biomarkers of Exposure

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Abstract

Two biomarkers of exposure to cigarette smoke, 4-aminobiphenyl-hemoglobin (Hb) adducts and aromatic DNA adducts in lymphocytes, were determined from a population of 55 smokers and 4 nonsmokers. The levels of these adducts were related to daily cigarette consumption and also to (calculated) tar and nicotine intake. The Hb adduct levels seemed to correspond best to the number of cigarettes (cig) smoked, but at a cigarette consumption of >30 cig/day, a saturation effect was observed. Lymphocytic DNA adducts also correlated well with cigarette and tar consumption; for this type of adduct, a saturation level was reached at a dose of ~15–20 cig/day. From a subpopulation, a second sample was obtained after 2 months, and the adduct levels were compared with their initial adduct levels. Strong correlations were found between the first and second DNA adduct measurements ($r = 0.84$). In another subpopulation, resampling was performed after 6 months. No correlation between DNA adduct levels in the first and last samples was found, but 4-aminobiphenyl Hb adduct levels were strongly correlated ($r = 0.78$), the absolute quantities measured being comparable (paired t test: $t = -1.27$, $P = 0.22$, $n = 15$). We found no influence of *GSTM1* and *NAT2* polymorphisms on Hb adduct formation and of *GSTM1* polymorphism on aromatic DNA adduct formation. A significantly lower aromatic DNA adduct level was observed for intermediate acetylators when compared to slow acetylators.

Introduction

Various studies on the relationship between 4-ABP-Hb[2] adduct levels and smoking behavior have demonstrated enhanced 4-ABP-Hb levels for smokers *versus* nonsmokers (1, 2) and significant correlations between 4-ABP-Hb adduct levels and number of cig smoked (3), type of tobacco used (3, 4), and acetylator phenotype of smokers (5, 6). Relationships between both 4-ABP-Hb adduct levels and 4-ABP-DNA adduct levels in urothelial cells and the occurrence of bladder cancer have been established, indicating a causal role for tobacco smoking (7, 8).

Polycyclic aromatic hydrocarbons constitute a class of compounds that is also associated with cigarette smoking-related carcinogenesis. Occasionally, polycyclic aromatic hydrocarbon-DNA adducts are used as indicators for the biologically effective dose (9). In organs that are susceptible to cigarette smoking-induced diseases (*e.g.*, lung, cervix, larynx, and heart), relationships have been found between aromatic DNA adduct levels and cigarette smoking. In organs that are not considered to be associated with cigarette smoke-induced diseases (*e.g.*, breast and liver), no such relationships have been observed (10). Because susceptible tissues can rarely be obtained, more accessible sources of exposed DNA have been used in various human studies. For instance, DNA of peripheral WBCs (11, 12), of isolated subpopulations of WBCs (13–15) and of pulmonary alveolar macrophages obtained by bronchoalveolar lavages (16, 17) have been analyzed. In general, lymphocyte DNA adduct levels are found to be higher in smokers than in nonsmokers (15).

Here, we describe a population of 55 smoking and 4 nonsmoking volunteers, who donated blood samples for the investigation of 4-ABP-Hb adduct levels and aromatic DNA adduct levels in peripheral lymphocytes. The studies were performed to sort out which of these two biomarkers best reflects the virtual smoking behavior of the subjects. Toward this aim, not only the number of cig smoked but also properties of cig, *e.g.*, nicotine and tar contents, were evaluated.

Furthermore, we investigated the possibility of saturation of adduct formation for these two types of adducts at high exposure levels.

Although both acetylator phenotype (5, 6) and glutathione *S*-transferase *M1* genotype (18) have been related to 4-ABP detoxification reactions and adduct levels, the observed relations between 4-ABP levels and these polymorphisms are not unequivocal. In fact, the composition of the population used may strongly influence the results (5, 6, 18). The genetic polymorphisms *GSTM1* and *NAT2* have also been related to the formation of aromatic DNA adducts (19, 20). Therefore, we

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[2] The abbreviations used are: 4-ABP, 4-aminobiphenyl; Hb, hemoglobin; cig, cigarettes; 4'F-ABP, 4'-fluoro-4-ABP; GC, gas chromatograph; MS, mass spectrometer; r_s, Spearman's rank correlation coefficient.

also investigated the role of these genetic polymorphisms on the adduct formation in our study population.

Materials and Methods

Study Population. The study population consisted of 36 female and 19 male cigarette-smoking volunteers with a mean age of 42 ± 9 years and 4 nonsmoking volunteers (2 males and 2 females with a mean age of 25 ± 2 years), who participated so that background adduct levels could be assessed. Questionnaires were used to obtain information on smoking, medication, possible occupational exposure, and so on. All questioned persons were included in the study. No confounding due to occupational exposure or medication was anticipated. The study was approved by the medical ethical commission of the Netherlands Cancer Institute, and informed consent was obtained from all volunteers.

Isolation of Hb. Peripheral blood was sampled by venipuncture into EDTA tubes, and erythrocytes were isolated according to the method described by Bryant *et al.* (1), with minor modifications. After centrifugation, the packed RBCs were washed twice with 0.9% saline and frozen at −80°C overnight. After thawing, the lysation of the erythrocytes was completed by adding three volumes of ice-cold deionized water. After 20 min at 0°C, the solution was buffered with phosphate at pH 6.6. The cell membranes were removed by centrifugation at 4°C. The remaining Hb solution was dialyzed against deionized water for 48 h at 4°C to remove ions and molecules with a molecular weight below M_r 12,000. The Hb concentration of the dialysate was determined using Drabkin's method (Sigma kit 525a), and the dialysate was stored at −20°C until further use.

Isolation and Derivatization of 4-ABP. Twenty ml of the dialysate were spiked with 1 ng of 4'F-ABP and incubated for 30 min at room temperature. 4-ABP was released from the Hb by the addition of 0.2 ml of 10 N NaOH and an incubation of 2.5 h at room temperature. The dialysate was extracted twice with 20 ml of hexane; the resulting foamy layer between the aqueous and hexane layers was broken down by the addition of 50 μl of ethanol, followed by centrifugation. The combined hexane layers were dried over anhydrous Na_2SO_4-$MgSO_4$, collected in deactivated glass vessels (21), and evaporated to a volume of ~2 ml. 4-ABP and 4'F-ABP were derivatized by the addition of 5 μl of triethylamine in hexane (1:5) and 3 μl of pentafluoropropionic anhydride, followed by a waiting time of 2 h at room temperature (22). The hexane was then evaporated, and the residue was redissolved in 20 μl of dichloromethane.

Determination of 4-ABP. The 4-ABP/4'F-ABP solution in dichloromethane (0.5 μl) was splitlessly introduced into the GC-MS, and ions at *m/z* 295 and *m/z* 313, from 4-ABP and 4'F-ABP, respectively, were measured using negative ion chemical ionization mass spectrometry under selected ion-monitoring conditions (1). The GC-MS instrument existed of a HP 5890 Series II capillary GC (Hewlett Packard, Portland, OR) coupled to a Jeol SX 102A double-focusing MS of reversed geometry (JEOL Ltd., Tokyo, Japan). The GC was operated at an injector temperature of 260°C, using a 25-m, inside diameter 0.32 mm, film thickness 0.25 μm fused silica SGE BPX35 column (SGE Inc., Austin, TX). The column temperature was 60°C for 2 min and then rose at 30°C/min to 320°C. Helium was used as the carrier gas at a flow rate of 1 ml/min. The MS was operated at an ion source temperature of 230°C, an ionizing energy of 200 eV, and an accelerating potential of 10,000 V; methane was used as the reaction gas. The 4-ABP concentration in Hb was calculated using the known Hb and internal standard concentrations of the dialysate. The mean interassay variation and SE for the 4-ABP determination starting from duplicate blood samples was 22.0 ± 3.5% (n = 5); duplicate measurements starting from the same dialysate showed a variation of 10.4 ± 5.2% (n = 4). This variation could almost entirely be explained by duplicate injections from the same extract into the GC-MS system: variation = 9.2 ± 0.8% (n = 82). The detection limit was <1 pg of 4-ABP/g of Hb.

Isolation of DNA. Lymphocytes were isolated by centrifugation on lymphoprep according to Bøyum (23), washed with PBS, and stored at −20°C until DNA isolation. The lymphocytes were lysed with 2.5 ml of SDS-NEP (75 mM NaCl, 25 mM EDTA, 50 μg/ml proteinase K, and 1% SDS) and incubated overnight at 37°C. DNA was extracted with phenol, chloroform, and *iso*-amylalcohol (25:24:1, v/v) and chloroform and *iso*-amylalcohol (24:1). The DNA was precipitated with 2 volumes of cold ethanol after addition of 0.03 volumes of 3 M sodium acetate (pH 5.3) and washed with 70% ethanol. The DNA was dissolved in 5 mM Tris. RNase T1 (50 units/ml) and RNase A (100 μg/ml) were added, followed by 30 min of incubation at 37°C. The solutions were extracted with chloroform and *iso*-amylalcohol (24:1). DNA was precipitated from the aqueous phase with ice-cold ethanol, washed with 70% ethanol, and dissolved in 2 mM Tris (pH 7.4). Concentration and purity were determined spectrophotometrically by $A_{230\,nm}$, $A_{260\,nm}$, and $A_{280\,nm}$, and the final volume was adjusted to achieve a DNA concentration of 2 mg/ml.

^{32}P-Postlabeling. The ^{32}P-postlabeling assay, which was used to determine aromatic DNA adducts, was performed as described by Reddy and Randerath (24), with some modifications. DNA (10 μg) was digested using micrococcal endonuclease (0.4 unit) and spleen phosphodiesterase (2.8 μg) for 3 h at 37°C. Subsequently, half of the digest was treated with nuclease P1 (6.3 μg) for 40 min at 37°C. The modified nucleotides were labeled with [γ-^{32}P]ATP (50 μCi/sample) by incubation with T4-polynucleotide kinase (5.0 units) for 30 min at 37°C. The radiolabeled adduct nucleotide biphosphates were separated by chromatography on PEI-cellulose sheets (Machery Nagel, Düren, Germany). The following solvent systems were used: D1, 1 M NaH_2PO_4, pH 6.5; D2, 8.5 M urea and 5.3 M lithium formate, pH 3.5; D3, 1.2 M lithium chloride, 0.5 M Tris, and 8.5 M urea, pH 8.0; D4, 1.7 M NaH_2PO_4, pH 6.0. More details are published elsewhere (25). Each sample was analyzed in three independent experiments; the mean interassay variation (and SE) was 19.1 ± 2.4% (n = 53). The detection limit was 0.1 adduct/10^8 nucleotides.

***GSTM1* Polymorphism.** The glutathione *S*-transferase *M1* genotype of the smokers was determined using a PCR-based assay, as described by Zhong *et al.* (26). PCR was carried out in a total volume of 100 μl, containing 0.5 μg of genomic DNA, 200 μg of each dNTP, 10 μl of DSMO, and 10 μl of PCR buffer containing 15 mM $MgCl_2$. Three PCR primers were used: 200 ng of the 24-mer primer P1 and 100 ng of both 20-mer primers P2 and P3 (see Ref. 26 for structures). After a 5-min denaturation time at 94°C, 2 units of *Taq* DNA polymerase (Pharmacia Biotech, Roosendaal, the Netherlands) were added, and the reaction was subjected to 40 cycles of amplification. A 10-μl aliquot of the PCR product was analyzed on a 3% agarose gel for the presence of DNA bands of 160 bp (control) and 232 bp (*GSTM1*).

***NAT2* Polymorphism.** The *N*-acetyltransferase 2 genotype of 35 smokers was determined using standard procedures (27). PCR was carried out in a total volume of 100 μl, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.0 μg of genomic DNA,

0.2 mM each dNTP, 1 μg of each primer (see Ref. 27 for structures), and 2.5 units of *Taq* DNA polymerase. This mixture was heated to 94°C to denaturate DNA and subjected to 30 cycles of amplification. Digestion of 2 μg of the PCR product was carried out in a volume of 40 μl, using the appropriate buffers and the restriction enzymes *Msp*I and *Kpn*I, *Bam*HI and *Taq*I, or *Dde*I. Digested samples were loaded on a 5% acrylamide gel containing ethidium bromide, run for 16 h at 30 V, and visualized and photographed under UV light. Classification of the subjects by fast, intermediate, and slow phenotype was derived from the genotype data (28).

Statistics. Linear regression analysis was used to determine correlations between smoking parameters and macromolecular adduct levels (4-ABP-Hb adducts and DNA adducts). In addition, nonparametric Spearman's rank correlations were calculated because of observed nonlinearity at higher smoking doses (saturation effect). Comparison of the groups of smokers with the nonsmokers was performed using the nonparametric Mann-Whitney *U* test. This test was also used to compare the groups that were classified based on genetic polymorphisms. Adduct levels measured in samples that were collected 2 and 6 months after the first sampling were compared with the levels found in the first samples using correlation analysis and the paired *t* test.

Results

The 4-ABP-Hb adduct levels of the smokers showed no significant correlation with age or sex of the subjects or with duration of smoking. In this respect, the results of the 4-ABP-Hb measurements are comparable with those of the lymphocytic aromatic DNA adducts, which have been published separately (25).

Comparison of the 4-ABP-Hb adduct levels in smokers *versus* nonsmokers, using the Mann-Whitney *U* test, demonstrated significant different levels ($P = 0.001$): for nonsmokers, the mean (± SE) adduct level was 57 ± 9 pg/g of Hb ($n = 4$); for smokers, it was 202 ± 11 pg/g of Hb ($n = 55$). Using the Mann-Whitney *U* test to compare the lymphocytic aromatic DNA adduct levels, these levels appeared significantly different ($P = 0.005$) for smokers ($n = 55$), compared to nonsmokers ($n = 3$). The mean number of adducts (± SE) was 1.62 ± 0.10 adducts/10^8 nucleotides in smokers and estimated at 0.05 adducts/10^8 nucleotides in nonsmokers (half-value of detection limit).

In one subgroup of smoking volunteers, a second sample was obtained 2 months after the first sampling; in another group, this was done after 6 months. Within-person repeatability of Hb and DNA adducts was examined using correlation analysis. A paired *t* test was used to test the hypothesis that the average difference between first and second observations was 0. The results of the correlation analysis and the *t* test are presented in Table 1. For repeated DNA adduct measurements after 6 months, the hypothesis was rejected. The results of the repeated measurements are shown in Fig. 1. There are many different kinds of cig, notably with regard to nicotine and tar contents and, also, the presence of a filter. To address which of these differences might influence the internal dose of the tobacco smoke carcinogens, a possible relationship of these cigarette properties with the biomarkers was investigated. No differences between plain and filtered cig with regard to both adduct types could be established. The potential daily intake of nicotine and tar was calculated from the number of cig smoked and the nicotine and tar contents per cigarette. For both 4-ABP-Hb adduct levels and aromatic DNA adduct levels, Spearman's rank correlations with daily cigarette consumption and daily nicotine and tar intake were calculated. Significant correlations between 4-ABP-Hb and number of cig ($r_s = 0.38$, $P = 0.004$, $n = 59$) and between 4-ABP-Hb and tar ($r_s = 0.29$, $P = 0.03$, $n = 59$) were found (smokers and nonsmokers). The same dataset was analyzed using linear regression analysis. A significant relationship between the number of cig/day smoked and 4-ABP-Hb levels was found ($R^2 = 0.18$, $P = 0.004$, $n = 59$).

Table 1 Repeatability of adduct level determinations

	No. of samples	Correlation (r)	Paired t	P (two-tailed)
4-ABP-Hb adducts				
Repeated sampling after 2 months	9	0.62	0.33	0.75
Repeated sampling after 6 months	15	0.78	−1.27	0.22
Aromatic DNA adducts				
Repeated sampling after 2 months	8	0.84	1.21	0.27
Repeated sampling after 6 months	16	−0.29	−2.75	0.02

For lymphocytic aromatic DNA adduct levels, no Spearman's rank correlation with daily cigarette consumption was found. However, in the subpopulation of subjects smoking <30 cig per day, a correlation was found ($r_s = 0.41$, $P = 0.01$, $n = 40$). Similarly, strong positive correlations between DNA adduct formation and nicotine intake ($r_s = 0.30$, $P = 0.02$, $n = 58$) and tar intake ($r_s = 0.33$, $P = 0.01$, $n = 58$) were found. Linear regression analysis shows weak but significant correlations between the DNA adduct levels and the number of cig/day ($R^2 = 0.14$, $P = 0.004$, $n = 58$), nicotine intake ($R^2 = 0.09$, $P = 0.02$, $n = 58$), and tar intake ($R^2 = 0.10$, $P = 0.01$, $n = 58$).

Because both 4-ABP-Hb adducts and aromatic DNA adducts appeared to be related to at least one of the smoking parameters, we also investigated a potential correlation between these two parameters. A significant correlation between the two types of adducts was found ($r_s = 0.30$, $P = 0.02$, $n = 58$).

In a previous paper (25), we reported the relationship between the number of cig smoked per day and adduct-forming efficiency in lymphocytes: a saturation effect at cigarette numbers >20 was observed. From the present data, similar effects were observed; based on 55 subjects, negative correlations were found between adduct-forming efficiency and smoking parameters (Table 2). To determine whether the decline of the adduct/dose ratio occurs above a particular dose or is a gradual process, the relationship between mean doses and mean adduct levels was calculated for increasing doses, *i.e.*, for 0, 0–5, 0–10, and 0–15 cig/day and so on, the mean cigarette consumption was related to the mean adduct level. These relationships are shown in Fig. 2. To describe these relationships mathematically, a third- or higher-order polynomial curve would be needed to fit the data. However, a biological basis for such a high-order dose-response relationship is not opportune. For 4-ABP-Hb adducts, a positive linear correlation between mean dose and mean adduct level was found for 0–30 cig/day ($R^2 = 0.992$, $P = 0.0001$). Inclusion of data from the higher cigarette-consuming population led to a substantially less steep relationship ($R^2 = 0.942$, $P = 0.006$). At this high dose, 4-ABP-Hb adduct levels appear to be almost independent of the number of cig smoked. Also, a positive linear correlation was found for lymphocytic aromatic DNA adducts and the number of cig smoked. However, this correlation existed for numbers of cig, varying from 0–15/day ($R^2 = 0.998$, $P = 0.0009$). Inclusion of the results of the subjects smoking >15 cig/day also led to a less steep correlation: $R^2 = 0.907$, $P = 0.0001$. These adduct

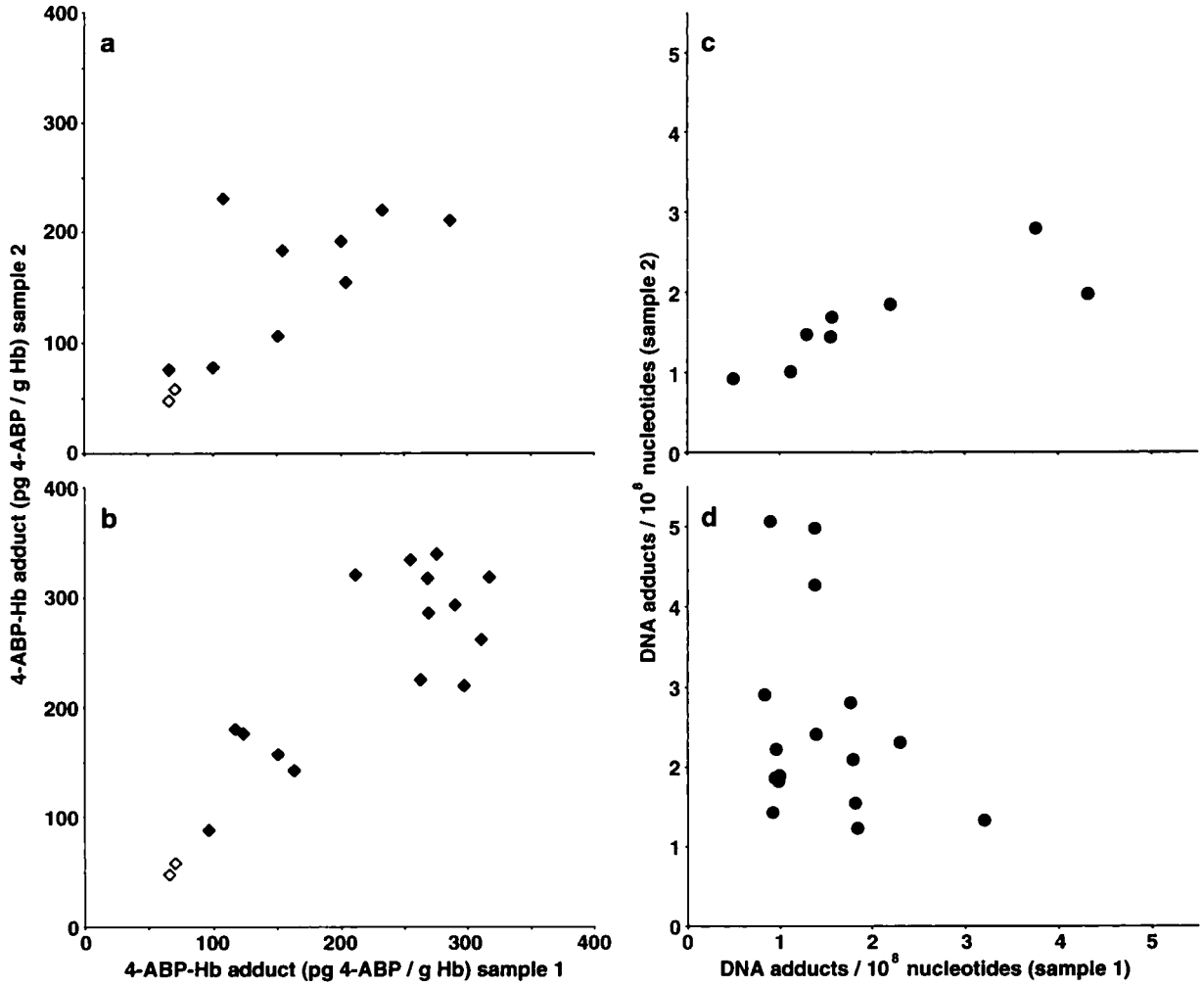

Fig. 1. Correlations of adduct levels of repeated measurements. *a*, 4-ABP-Hb (2-month interval, $r = 0.62$, $n = 9$). *b*, 4-ABP-Hb (6-month interval, $r = 0.78$, $n = 15$); *c*, lymphocytic aromatic DNA adducts (2-month interval, $r = 0.84$, $n = 8$). *d*, lymphocytic aromatic DNA adducts (6-month interval, $r = -0.29$, $n = 16$); ◇ data from a nonsmoker, not used for the calculated correlations.

Table 2 Spearman rank correlations between daily exposure and efficiency of adduct formation

Adduct level per unit of exposure	Total exposure per day to[a]	r_s	P
4-ABP-Hb adducts	cig	−0.64	0.0001
	Nicotine	−0.87	0.0001
	Tar	−0.87	0.0001
Aromatic DNA adducts	cig	−0.58	0.0001
	Nicotine	−0.71	0.0001
	Tar	−0.70	0.0001

[a] Nicotine and tar levels are in mg/day.

levels also did not appear to be significantly associated with the number of cig smoked. Between the mean tar consumption and 4-ABP-Hb and DNA adduct levels, no significant linear relationships were found; both relationships can be described using a third-order polynomial regression model. However, a biological explanation for such a relationship is not available. From the graphics in Fig. 3, it may be concluded that there was a gradual saturation effect.

No differences in the levels of both 4-ABP-Hb adducts and lymphocytic aromatic DNA adducts related to *GSTM1* polymorphism were found. The acetylator genotype was determined from 35 subjects, and from the genotype, the phenotype was deduced. Twenty-one subjects (60%) were of the homozygous slow acetylator genotype. The remaining 14 subjects (40%) were of the heterozygous intermediate acetylator genotype. No significant correlation between 4-ABP-Hb levels and acetylator phenotype was found. For aromatic DNA adducts in lymphocytes, however, a significant difference between intermediate and slow acetylators was detected ($P = 0.05$): slow acetylators, $n = 21$, adduct level = 1.69 ± 0.11 adducts/10^8 nucleotides (mean ± SE); and intermediate acetylators, adduct level = 1.36 ± 0.17 adducts/10^8 nucleotides.

Discussion

We found a correlation between 4-ABP-Hb adduct levels and cigarette consumption, a correlation that became even stronger when the population was limited to subjects smoking ≤30 cig per day. A similar effect was detected for aromatic DNA adducts in lymphocytes in relation to daily cigarette consump-

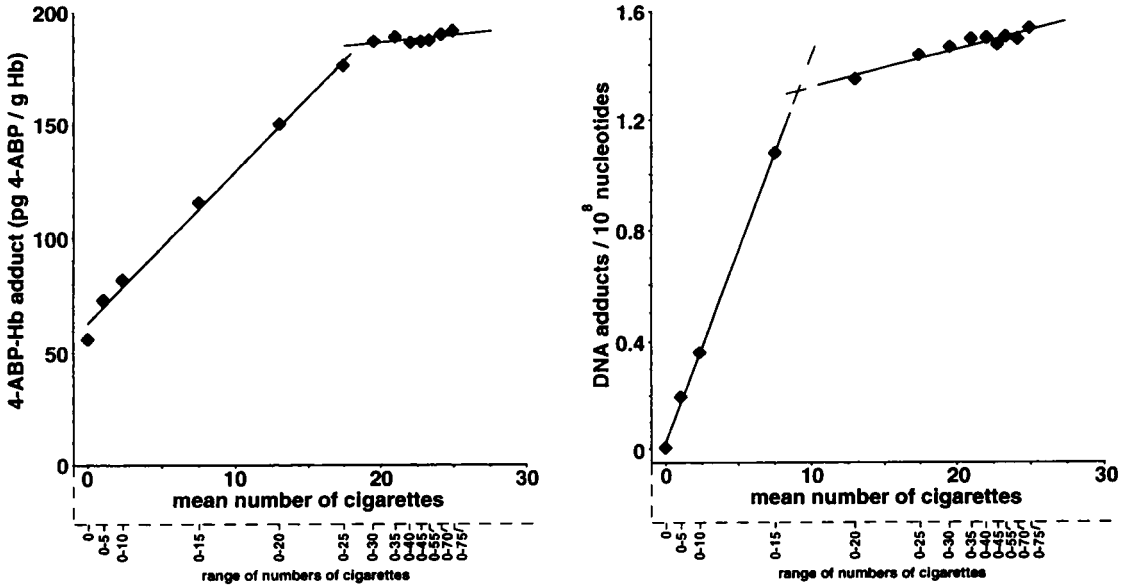

Fig. 2. Mean cigarette consumption in relation to mean adduct formation. Starting with the nonsmokers' data, the mean consumption for subjects smoking 0, 0–5, 0–10, and 0–15 cig/day and so on is related to the mean adduct levels of these groups; without a saturation effect, a linear relationship would be anticipated. *Left*, relationship for 4-ABP-Hb adducts; *right*, relationship for lymphocytic DNA adducts.

tion. The relationship between number of cig and adduct formation at low dose in combination with a decrease in adduct level:dose ratio at higher dose has been reported before for 4-ABP-Hb adducts (29) and for aromatic DNA adducts in lymphocytes (25). Apparently, the adduct-forming efficiency decreases at increasing exposure. This phenomenon might be explained by assuming a different smoking behavior at high doses, which would lead to an effectively stable (smoke) dose at high cigarette consumption, as suggested by others (30, 31). Another explanation might be a change in metabolic profiles at higher cigarette consumption (25, 29, 32). Tannenbaum (29) has substantiated this view with the statement that plasma cotinine steadily increases with increasing cigarette consumption. Our results, indicating cutoff points for the linear relationship between the number of cig and 4-ABP-Hb adducts at a consumption of 30 cig/day and for the relationship between the number of cig and lymphocytic aromatic DNA adducts at ~15 cig/day, demonstrate that, at least for the DNA adducts, the effect could not be explained by a different smoking behavior at high cigarette consumption. Perhaps, a DNA repair mechanism could play a role in this phenomenon. Furthermore, the rather sharp kinks in the mean dose-mean adduct level relationships that occur at 15 and 30 cig per day for DNA adducts and 4-ABP-Hb adducts, respectively, support the idea of different metabolic processes at higher cigarette consumption (Fig. 2).

Because both dosimeters, 4-ABP-Hb adducts and aromatic DNA adducts, appeared to be related to at least one of the smoking parameters, we also investigated a potential correlation between these two parameters. Although the metabolic transformation of the precursors of both types of adduct is not necessarily the same and, therefore, no causal relation is to be expected, the common source of these precursors, *i.e.*, tobacco smoke, might lead to a quantitative relationship between these two markers. Although a significant correlation between these two adducts was found (R^2 = 0.07, n = 58, P = 0.04), the variance was high, as can be seen from the small R^2 value. Therefore, it is concluded that the correlation between these two markers is mainly supported by the overall relationships between smoking parameters and adduct levels.

Determination of the acetylator genotype showed that 21 subjects (60%) were of the homozygous slow acetylator genotype, which matches exactly the results found for a German population (28). According to literature data (28), the heterozygous fraction should be ~32% and the homozygous (fast acetylator genotype) fraction should be 8%, which would correspond to 2 or 3 of 35 subjects. Apparently, the number of subjects sampled here is too small to match this statistically expected distribution. An influence of *N*-acetyltransferase phenotype (rapid acetylators *versus* slow acetylators) on 4-ABP-Hb adduct formation has been reported (5, 6, 33, 34), but in this study, we did not find such an effect in relation to the acetylator genotype. However, the differences between the groups of slow and rapid acetylators in one of these studies (6) were not significant, whereas the effects reported in another study (5) were only marginally significant (P = 0.046). In one study, significantly lower 4-ABP-Hb levels for rapid acetylators were accompanied by higher 4-ABP-Hb levels for heavy smoking rapid acetylators (34). Possibly, differences in the quality and variety of tobacco used in the various studies; differences in regional genetic and, therefore, metabolic properties of the subjects; and differences in classification of the subjects into slow, intermediate, and rapid acetylators (phenotyping *versus* genotyping), eventually in combination with varying conditions and confounders between the studies, can explain these differences in results. For the difference between the DNA adduct levels of slow and intermediate acetylators, our study fails to provide a mechanistic explanation. Yet unidentified aromatic components of the cigarette smoke might be subject to acetylation; on the other hand, the correlation observed might merely be coincidental.

Although blood and urine nicotine and cotinine levels only reflect recent exposure to tobacco smoke, DNA adducts and 4-ABP-Hb adducts could be expected to represent a steady-

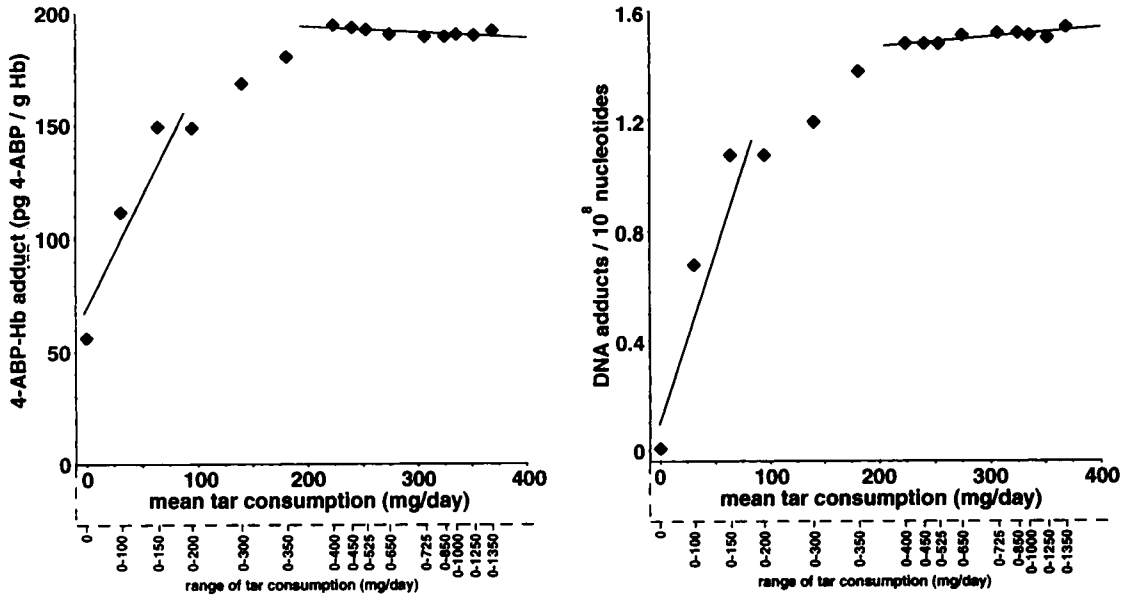

Fig. 3. Mean daily tar consumption in relation to mean adduct formation. Starting with the nonsmokers' data, the mean consumption for subjects smoking cig containing 0, 0–100, 0–150, and 0–200 mg of tar/day and so on is related to the mean adduct levels of these groups; without a saturation effect, a linear relationship would be anticipated. *Left*, relationship for 4-ABP-Hb adducts; *right*, relationship for lymphocytic DNA adducts.

state situation, reflecting long-term continuous exposure. For the 4-ABP-Hb adducts, this view is confirmed in this study. Resampling of a subpopulation of smokers 2 months after the first sampling only achieved a poor correlation between the first and second samples ($r = 0.62$), but this may be due to the small number of samples. Resampling of a larger subpopulation of smokers 6 months after the first sampling, however, led to an improved correlation in adduct levels ($r = 0.78$) at a constant level. For lymphocytic DNA adducts, the long-term reproducibility is much less pronounced. An initial correlation of DNA adduct levels that was found after 2 months (albeit at a lower level) could not be reproduced after 6 months. Probably, seasonal variations (25) combined with an intraindividual variation in numbers of lymphocytes (for instance, due to infections), annuls such a potential correlation.

It is concluded that, for the biological effect monitoring of smoking behavior of long-term smokers, the determination of 4-ABP-Hb adduct levels is superior to the determination of lymphocytic aromatic DNA adducts.

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