ERRATUM

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Nicotine & Tobacco Research Volume 8, Number 4 (August 2006) 600–622

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Review

Biomarkers to assess the utility of potential reduced exposure tobacco products

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[Received 10 December 2004; accepted 30 June 2005]

To date, we have no valid biomarkers that serve as proxies for tobacco-related disease to test potential reduced exposure products. This paper represents the deliberations of four workgroups that focused on four tobacco-related heath outcomes: Cancer, nonmalignant pulmonary disease, cardiovascular disease, and fetal toxicity. The goal of these workgroups was to identify biomarkers that offer some promise as measures of exposure or toxicity and ultimately may serve as indicators for future disease risk. Recommendations were based on the relationship of the biomarker to what is known about mechanisms of tobacco-related pathogenesis, the extent to which the biomarker differs among smokers and nonsmokers, and the sensitivity of the biomarker to changes in smoking status. Other promising biomarkers were discussed. No existing biomarkers have been demonstrated to be predictive of tobacco-related disease, which highlights the importance and urgency of conducting research in this area.

Introduction

Biomarkers for disease risk are critical in the assessment of potential reduced exposure products (PREPs; Stratton, Shetty, Wallace, & Bondurant, 2001). Because of the long exposure time necessary to determine the effects of PREPs on actual harm and the use by smokers of multiple products for different amounts of time, short-term proxies for disease that can be used in laboratory studies, clinical trials, and population studies are necessary. Biomarkers can be classified as a measure of (a) chemical exposure, that is, a direct or indirect measure of a tobacco-derived constituent or metabolite, that ideally can provide a quantitative estimate of tobacco exposure; (b) toxicity, including biologically effective dose, that is, "the amount that a tobacco constituent or metabolite binds to or alters a macromolecule either in target or surrogate tissue" (Stratton et al., 2001); (c) injury or potential harm, that is, "a measurement of an effect due to exposure; these include early biological effects, alterations in morphology, structure or function, and clinical symptoms consistent with harm" (Stratton et al., 2001); and (d) direct measures of health outcome. Genetic biomarkers for disease susceptibility also exist that may play a significant role in whether or not a smoker develops a disease.

The criteria for evaluating the utility of a biomarker in determining the effects of PREPs have been described in the Institute of Medicine report *Clearing the Smoke* (Stratton et al., 2001) and elsewhere (Shields, 2002). These criteria include target tissue and outcome relationship (e.g., the extent to which the biomarker reflects a measurement of pathogenesis), dose-response data for harm (e.g., the relationship between amount of tobacco smoke or constituent exposure and biomarker of potential harm), dose-response data for harm reduction (e.g., predictive validity of the biomarker, that

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ISSN 1462-2203 print/ISSN 1469-994X online
DOI: 10.1080/14622200600858166

is, change in a biomarker is associated with change in disease risk), specificity (e.g., biomarkers that are specific for exposure to tobacco toxicants), sensitivity (i.e., the degree of detection), and reproducibility (e.g., intrasubject reliability).

For the purposes of this paper, we describe potential biomarkers of exposure or toxicity related to four disease states or adverse health outcomes: Cancer, neoplastic pulmonary disease, cardiovascular disease, and fetal toxicity. Some of the biomarkers overlap across disease states. To facilitate writing this paper, a conference, sponsored by the National Cancer Institute, the National Institute on Drug Abuse, the National Institute on Alcohol Abuse and Alcoholism, and the Centers for Disease Control and Prevention, was held in February 2004 to address methods and biomarkers to assess PREPs (Hatsukami et al., 2005). In addition to methods workgroups, biomarker workgroups were established to address each of these disease areas. Each workgroup (see acknowledgments for members of the respective workgroups) was asked to identify biomarkers based on their mechanistic relationship to pathogenesis and evidence showing (a) differences between smokers and nonsmokers; (b) change as a consequence of cessation; (c) a dose-response relationship between extent of exposure and level of the biomarker; and (d) change as a result of tobacco use reduction.

The evaluation of predictive validity of these biomarkers is critical in determining their utility in assessing PREPs. Although we have a good understanding of the mechanisms associated with tobacco-related disease, the current state of our science on biomarkers is limited and minimal information is available on the dose-response relationship to harm of most, if not all, of the existing biomarkers. Furthermore, changes in biomarkers according to changes in smoking status does not provide information on the extent of quantitative change needed to reduce disease risk, particularly in an individual with a long history of tobacco use. With these caveats in mind, based on the existing data, the workgroup for each disease state made recommendations of biomarkers that can be useful for studying PREPs with the clear understanding that none of these biomarkers indicate reduced disease risk. Recommendations also were made for promising biomarkers that can be considered in future research for reliability and validity testing.

Cancer-related biomarkers

The cancer-related biomarkers are summarized in Table 1. They are divided into two categories: Chemical biomarkers and cellular biomarkers. Chemical biomarkers are specifically related to tobacco carcinogens and are reflective of exposure but not necessarily harm. Cellular biomarkers are biomarkers of biologically effective dose, toxicity, and injury. Cigarette smoke contains over 60 known carcinogens, and unburned tobacco contains over 15 (Hecht, 2003). Carcinogens are mainly responsible for the cancer-causing effects of tobacco products, although irritation, inflammation, tumor promotion, and cocarcinogens probably play a role. Some tobacco carcinogens bind directly to DNA whereas most require enzymatic activation. The resulting covalent binding products, called DNA adducts, are central to the carcinogenic process and most likely cause the multiple mutations found in specific growth control genes in tumors. The chronic barrage of DNA damage from years of use of tobacco products is consistent with the multiple genetic changes observed in tobacco-associated cancers (Hecht, 2003). Chemical biomarkers measure carcinogen uptake and some measure metabolic activation and binding to DNA. Cellular biomarkers measure biological effects associated with cancer. These include genetic damage of various types and other cellular changes.

Within each category, the biomarkers are listed roughly in order of their present utility in studies evaluating new products with the most useful ones listed first. Utility is based on considerations of analytical specificity and sensitivity and relationships of the biomarker to tobacco product use. The latter category is particularly important in the present context and is divided into four subcategories illustrated in Table 1.

Among the chemical biomarkers, we consider 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronides (NNAL-Glucs) in urine and aminobiphenyl/aromatic amine hemoglobin (Hb) adducts to be the most useful at the present time for studies evaluating new products. NNAL and NNAL-Glucs are metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK; Hecht, 1998). NNK is widely considered to play an important role as a cause of lung cancer in smokers and also may be involved in the etiology of several other tobacco-related cancers (Hecht, 2003). NNAL and NNAL-Glucs are found in the urine of all people who use tobacco products but never in nonusers unless they are exposed to environmental tobacco smoke (Hecht, 2002). In the latter case, levels are one-twentieth or less than those found in the urine of smokers or users of smokeless tobacco products. The specificity of NNAL and NNAL-Glucs to tobacco exposure and their direct relationship to a lung carcinogen, NNK, are highly attractive features of these biomarkers. Currently

Table 1. Cancer-related biomarkers.

Biomarker	Measurement of	Relation to tobacco product use			
		Difference: users vs. nonusers	Change with cessation	Dose response with use	Change with reduced use
1. Chemical biomarkers					
NNAL and NNAL-Glucs in urine	Carcinogen (NNK) uptake	x (Carmella et al., 1993)	x (Hecht et al., 1999)	x (Joseph et al., 2005)	x (Hecht, Murphy et al., 2004)
3-Aminobiphenyl, 4 aminobiphenyl, and other aromatic amine-Hb adducts	Carcinogen (aromatic amines) uptake plus metabolic activation	x (Castelao et al., 2001)	x (Maclure et al., 1990; Skipper & Tannenbaum, 1990)	x (Castelao et al., 2001; Skipper & Tannenbaum, 1990)	
1-Hydroxypyrene in urine	Carcinogen (PAH) uptake	x (Hecht, 2002)	x (Hatsukami et al., 2004)		x (Hecht, Carmella et al., 2004)
Trans, *trans*-muconic acid in urine	Carcinogen (benzene) uptake	x (Scherer et al., 1998)			
S-phenylmercapturic acid	Carcinogen (benzene) uptake	x (Hecht, 2002; Lin et al., 2004; Maestri et al., 2005; Melikian et al., 2002; Tharnpoophasiam et al., 2004)			
Benzene and other volatile organic carcinogens (VOCs) in exhaled air	Volatile organic carcinogens	x (IARC, 2004)		x (IARC, 2004)	
Ethylene oxide-Hb adducts	Carcinogen (ethylene oxide) uptake	x (Fennell et al., 2000)			
Other N-terminal valine adducts in Hb	Carcinogen uptake	x (Carmella et al., 2002)			
Cadmium and other metals in blood and urine	Carcinogen uptake	In part[a] (IARC, 2004)			
Acetaldehyde-DNA and protein adducts	Carcinogen uptake				
F_2-isoprostanes and oxidized proteins	Oxidative damage, inflammation[c]	x (J. D. Morrow et al., 1995; Pignatelli et al., 2001)			
8-OxoG or 8-oxo-dG in DNA or urine[b]	Oxidative damage, inflammation[c]	In part (Hecht, 2002; IARC, 2004)			
Mercapturic acids of acrolein and related compounds in urine	Toxin uptake and metabolism	In part (Hecht, 2002)			
Benzo[*a*]pyrene diol epoxide-DNA and Hb adducts	Carcinogen (BaP) uptake and metabolic activation	In part (Boysen & Hecht, 2003)			
NNK and NNN-DNA and Hb adducts	Carcinogen (NNK/NNN) uptake and metabolic activation	In part (IARC, 2004)			
Apurinic sites in DNA	DNA damage				
^{32}P-postlabelling of DNA	Carcinogen uptake and metabolic activation	x (IARC, 2004; Kriek et al., 1998; Phillips, 2002)	x (IARC, 2004; Kriek et al., 1998; Phillips, 2002)		
Immunoassays for DNA damage	Carcinogen (mainly PAH) uptake and metabolic activation	x (IARC, 2004; Kriek et al., 1998; Phillips, 2002)	x (IARC, 2004; Kriek et al., 1998; Phillips, 2002)		
2. Cellular biomarkers					
Urine mutagenicity	Mutagen uptake	x (IARC, 2004)	x (IARC, 2004)	x (IARC, 2004)	x (Benowitz et al., 1986)
Sister chromatid exchange in peripheral lymphocytes	DNA damage	x (IARC, 2004)	x (IARC, 2004)	x (Barale et al., 1998)	
Chromosomal aberrations and micronuclei frequency in lymphocytes	DNA damage	In part (IARC, 2004)			
HPRT mutant frequency in cultured lymphocytes	Gene mutations	In part (IARC, 2004)			

Table 1. *(Continued.)*

Biomarker	Measurement of	Relation to tobacco product use			
		Difference: users vs. nonusers	Change with cessation	Dose response with use	Change with reduced use
Bronchial metaplasia and dysplasia, sputum atypia	Preneoplastic changes	In part (Khuri et al., 2001; Lam et al., 1999; Lam et al., 2002; Prindiville et al., 2003)	In part (Khuri et al., 2001; Lam et al., 1999; Lee et al., 1994; Prindiville et al., 2003)		
Comet assay-DNA strand breaks	DNA damage	No consistent effect (Moller et al., 2000)			
Proteome differences	Effects on proteins				
Promoter methylation	Effects on gene expression				
Carcinoembryonic antigen	Inflammation[c]	In part (Ohwada et al., 1995; Stockley et al., 1986)			
Others (see text)	Inflammation				

Note. x, relation observed. [a]"in part", some studies support change in biomarkers by smoking status. [b]Published values may be unreliable due to unrecognized artifact formation. [c]Uncertainty exists over whether the biomarker is a measurement of inflammation.

available methods have high analytical specificity and sensitivity (Byrd & Ogden, 2003; Carmella, Han, Fristad, Yang, & Hecht, 2003). Differences between tobacco users and nonusers, relatively rapid decreases with cessation of tobacco use, dose-response relationships, and changes with reduced smoking are all established features of the NNAL and NNAL-Glucs biomarkers (Table 1). Hb adducts of aromatic amines are well established biomarkers of their uptake and metabolic activation (Castelao et al., 2001; Skipper & Tannenbaum, 1990). One of these compounds, 4-aminobiphenyl, is a known human bladder carcinogen and a likely cause of bladder cancer in smokers (Hecht, 2003). Although aromatic amines are not specific to cigarette smoke exposure, levels of 4-aminobiphenyl and, in particular, 3-aminobiphenyl-Hb adducts are considerably higher in smokers than in nonsmokers (Castelao et al., 2001). Adduct levels decrease with cessation and increase with cigarette consumption (Table 1). Highly sensitive and specific analytic methods are available for quantitation of these adducts.

1-Hydroxypyrene in urine, a biomarker of uptake of carcinogenic polycyclic aromatic hydrocarbons (PAHs), has been widely used in studies of smokers as well as in occupational studies (Jongeneelen, 2001). Considerable evidence indicates that PAHs are important causes of lung cancer and cancers of other tissues caused by cigarette smoking (Hecht, 2003). A drawback of this biomarker is its lack of specificity to tobacco exposure. Levels of 1-hydroxypyrene in urine are two to three times higher in smokers than in nonsmokers, with diet thought to contribute significantly to the levels in nonsmoker (Hecht, 2002). 1-Hydroxypyrene in urine also decreases with smoking cessation (Hatsukami et al., 2004). The benzene metabolite *trans*, *trans*-muconic acid is a widely used uptake biomarker for benzene, a known human leukemogen, and a quantitatively significant constituent of cigarette smoke (Scherer, Renner, & Meger, 1998). Like 1-hydroxypyrene, this biomarker is often elevated in smokers but lacks specificity to tobacco products (Scherer et al., 1998). Effects of smoking cessation on levels of *trans, trans*-muconic acid in urine have not been reported. *S*-Phenylmercapturic acid (*S*-PMA) is another biomarker of benzene exposure, which is elevated in smokers and appears to have higher specificity than *trans, trans*-muconic acid (Hecht, 2002; Lin, Tyan, Shih, Chang, & Liao, 2004; Maestri, Negri, Ferrari, Ghittori, & Imbriani, 2005; Melikian et al., 2002; Tharnpoophasiam, Kongtip, Wongwit, Fungladda, & Kitayaporn, 2004). Hb adducts of the carcinogens ethylene oxide, acrylamide, acrylonitrile, and of an unknown ethylating agent are higher in smokers than in nonsmokers but are not specific to tobacco use (Bergmark, 1997; Carmella et al., 2002; Fennell et al., 2000). Effects of smoking cessation have not been

reported. Several of the other chemical biomarkers listed in Table 1 similarly are elevated in smokers but either lack specificity to tobacco use or have not been developed fully enough to be considered as having high priority for current use in product evaluation studies.

Several entries deserve special comment. Hb adducts of NNK and *N*′-nitrosonornicotine (NNN) are tobacco specific and have the potential to measure uptake plus metabolic activation of these carcinogens. Robust and sensitive methods are available for their quantitation, but their levels are quite low and undetectable in many active smokers (Atawodi et al., 1998; Falter, Kutzer, & Richter, 1994; Hecht, Carmella, & Murphy, 1994). This creates practical problems when designing studies for evaluation of tobacco products. Similar considerations apply to benzo[*a*]pyrene diol epoxide-Hb and DNA adducts. Although these DNA adducts are undoubtedly important in cancer induction by tobacco products, they are difficult to detect even with highly sensitive methods, and many active smokers will not have detectable adduct levels (Boysen & Hecht, 2003). ^{32}P-Postlabelling and immunoassays have been widely used to quantify DNA adducts in smokers (International Agency for Research on Cancer [IARC], 2004; Kriek, Rojas, Alexandrov, & Bartsch, 1998; Phillips, 2002). These methods are highly sensitive and require only small amounts of DNA. Many studies have shown higher adduct levels in smokers than in nonsmokers. However, the methods have drawbacks. It is not clear what is being measured in the ^{32}P-postlabelling assays other than unspecified DNA-reactive material. They may be DNA adducts of hydrophobic compounds such as PAHs, but little definitive evidence supports this possibility. There can also be problems with quantitation, particularly with known adduct standards being unavailable. Immunoassays are sensitive and convenient, but problems exist with quantitation and specificity to particular PAHs.

Among the cellular biomarkers, detection of mutagens in urine with *S. typhimurium* strains has been the most widely applied. Many studies have demonstrated the dependency of urinary mutagenicity on cigarette smoking (IARC, 2004). Urinary mutagenicity could be considered as a chemical marker, because it is responding mainly to aromatic amines and their metabolites, and related compounds, that originate in cigarette smoke. The presence of these compounds in a smoker's urine causes a biological response in the test system, *S. typhimurium*, thus indicating their potential for DNA damage, but it is not clear that the damage also occurs in the smoker's bladder epithelium. A confounding factor in this assay that affects its specificity is diet, which can also affect urinary mutagenicity. Sister chromatid exchanges in peripheral lymphocytes are consistently elevated in smokers (IARC, 2004). This assay therefore provides a reasonably reliable measure of DNA damage in smokers and may be quite useful in studies of tobacco product effects. A limitation is that it is not clear which compounds in cigarette smoke cause sister chromatid exchanges. Data from other biological assays of genetic effects such as chromosomal aberrations, micronuclei, hypoxanthine guanine phosphoribosyl transferase (HPRT) mutations, and the Comet assay are much less consistent with respect to the effects of smoking (IARC, 2004; Moller, Knudsen, Loft, & Wallin, 2000).

Direct measurement of changes in the lungs of smokers, using modern bronchoscopy and sputum analysis techniques, may be the best approach to evaluating effects of tobacco products on lung cancer given that pulmonary metaplasia and dysplasia of the airway epithelium are known to be precursor lesions (Khuri et al., 2001; Lam et al., 1999, 2002; Lee et al., 1994; Prindiville et al., 2003). However, these methods are still invasive and somewhat subjective and will present significant problems with respect to participant recruitment.

In summary, NNAL and NNAL-Glucs in urine, aromatic amine-Hb adducts, urinary mutagenicity, and sister chromatid exchanges are presently the most practical biomarkers for use in studies evaluating new tobacco products. However, although the relationship of these biomarkers to cancer risk is highly plausible, in no case has it been demonstrated. Furthermore, even though measures of exposure to individual carcinogens or the presence of DNA adducts are useful tools for research, a difference in a single measure is not predictive of a difference in individual or population disease risk. To date, we have no comprehensive set of biomarkers of carcinogen exposure or biological effects as a predictive measure of the total carcinogenicity related to exposure to tobacco or tobacco smoke.

Nonmalignant pulmonary disease biomarkers

Cigarette smoking is associated with an increased incidence and severity of several nonmalignant lung diseases. These conditions have complex etiologies, and for the most part they occur in nonsmokers as well as smokers, although with reduced frequency and severity in nonsmokers. Available evidence does not permit a definitive overall pathogenesis of cigarette smoke-induced lung disease, but most of these tobacco-related diseases, such as chronic obstructive pulmonary disease (COPD), respiratory bronchiolitis, and pneumococcal infection, share features of increased inflammation or altered immune response. The relationship of smoking to

these disorders, therefore, has often been suggested to depend on the ability of cigarette smoke to initiate or amplify an inflammatory or immune response. Because of the complex pathophysiology of the various diseases, a large number of biomarkers have been explored. These biomarkers fall into several large categories: Measures of inflammation, alterations in tissue structure, and physiological alterations. No biomarkers have been established as specific for cigarette smoke-induced nonmalignant lung disease. Moreover, no markers have been validated as surrogate measures that can predict disease progression or response for any of the diseases related to smoking, with the exception of rate of progression of lung function decline, which is a marker for COPD risk. Finally, few of the markers have been studied specifically as they relate to smoking, smoking cessation, or harm reduction.

With this limited scientific literature in mind, Table 2 includes chemical and cellular as well as functional and directly observed biomarkers that have been reported to be altered in smokers versus nonsmokers. They are grouped, for convenience, by analytic method. Endobronchial biopsy is becoming an increasingly important means to assess the airways in patients with asthma and COPD (Hattotuwa, Gamble, O'Shaughnessy, Jeffery, & Barnes, 2002; Jeffery, Holgate, & Wenzel, 2003; Kavuru, Dweik, & Thomassen, 1999; Tashkin, 2002). Histological assessments, however, have been relatively limited in normal healthy smokers, and the many potential histological markers, which may eventually prove useful as biomarkers relevant for harm reduction, are not specifically included in Table 2 owing to the current lack of data.

The least invasive method to assess biomarkers in the lung is the analysis of exhaled breath. This is the most recently developed method, as obtaining samples free of oral contamination was difficult prior to the advent of specialized equipment. The technique is now readily applied and studies are being performed in a number of settings (Kharitonov & Barnes, 2001, 2002). Although a number of biomarkers have been reported to be increased in the exhaled breath condensate of smokers (Table 2.1), none are well validated. In addition, controversy exists about the most appropriate method to express data obtained from exhaled breath condensate (Effros et al., 2002).

Sputum analysis is only slightly more invasive than exhaled breath condensate collection. The samples, however, can be difficult to obtain and must be processed rigorously to yield suitable results. Analysis of inflammatory markers has been performed in sputum, either produced spontaneously or following induction with hypertonic saline (Fahy, Liu, Wong, & Boushey, 1993; Hargreave, 1999). Induced sputum analysis has also been applied to smokers, and one study specifically analyzed sputum specimens in ex-smokers (Hill, Bayley, Campbell, Hill, & Stockley, 2000) and another study following smoking cessation (Swan et al., 1992). Most readily assessed in sputum are cell numbers and differential counts. These studies have consistently demonstrated increased numbers of neutrophils and, in sufficiently large studies, increased eosinophils. Analyses of cytokines in the sputum and of specific measures of cellular activity have been performed, but these studies require specialized methods and some controversy exists about the best method to process specimens.

The nonsputum method with the longest experience for sampling the lung is bronchoalveolar lavage (BAL; Klech & Pohl, 1989; Linder & Rennard, 1988; Reynolds, 1987). Though invasive and relatively expensive, the procedure is reasonably well tolerated. It has been used to study most of the lung diseases associated with smoking. Much of the current understanding of the pathophysiology of these diseases derives, at least in part, from BAL studies. Any of the measures made as part of these studies could be a biomarker of biological response and potentially of injury related to smoking. BAL also has been used to assess changes following smoking cessation and with smoking reduction (Table 2.1). The most reliable biomarker of smoke exposure is an increase in the number of alveolar macrophages recovered. These cells are characteristically filled with pigment thought to be derived from the smoke and from the cellular debris caused by smoke-induced injury. The increased recovery of BAL macrophages corresponds well to the increased macrophages present in histological studies (Niewoehner, Kleinerman, & Rice, 1974). Decreased recovery of macrophages has been reported within a month of cessation (Skold, Hed, & Eklund, 1992). The pigment-laden macrophages persist after cessation for a much longer time (Agius, Rutman, Knight, & Cole, 1986; Skold, Hed et al., 1992). This persistence is thought to result from recycling of the pigmented material within the lung (Marques, Teschler, Guzman, & Costabel, 1997; Skold, Eklund, Hed, & Hernbrand, 1992). The total number of neutrophils also is reported to be increased in the lungs of smokers. However, the number of neutrophils increases less than the number of macrophages. As a result, in the lungs, the neutrophil percentage may decrease when expressed as a differential count. A large variety of cytokines and mediators have been assessed in BAL fluid. The application of proteomic and genomic methodology to specimens obtained from the lower respiratory tract by bronchoscopy or collection of sputum

Table 2. Nonmalignant lung disease biomarkers.

Biomarker	Measurement of	Relation to tobacco product use: Difference: users vs. nonusers	Change with cessation	Dose response with use	Change with reduced use
1. Chemical and cellular biomarkers					
Exhaled breath	Inflammation[a]				
Hydrogen peroxide	Oxidant	x (D. Nowak et al., 2001)			
Heptanal	Oxidative stress	x (Corradi, Rubinstein et al., 2003)			
Hexanal	Oxidative stress	x (Corradi, Rubinstein et al., 2003)			
Interleukin-1	Inflammatory cell activation	x (Garey et al., 2004)			
Interleukin-6	Inflammatory cell activation	x (Bucchioni et al., 2003; Carpagnano et al., 2003)			
Isoprostanes	Oxidative stress	x (Montuschi et al., 2000)			
Leukotriene B4	Inflammatory cell activation	x (Carpagnano et al., 2003)			
Malondialdehyde	Oxidative stress	x (Corradi, Rubinstein et al., 2003)			
Neutrophil chemotactic activity	Inflammation	x (Garey et al., 2004)			
Nitric oxide	Inflammation	x (Robbins et al., 1997)	x (Hogman et al., 2002; Robbins et al., 1997)		
Nitrite	Oxidative stress/ inflammation	x (Balint et al., 2001; Garey et al., 2004)			
Nitrate	Oxidative stress	x (Corradi, Pesci et al., 2003)			
Nitrite + nitrate	Oxidative stress	x (Balint et al., 2001)			
Nitrotyrosine	Oxidative stress/ inflammation	x (Balint et al., 2001)			
Nonanal	Oxidative stress	x (Corradi, Rubinstein et al., 2003)			
S-Nitrosothiols	Oxidative stress	x (Balint et al., 2001)			
Thiobarbituric acid reactive material	Oxidative stress	x (D. Nowak et al., 2001)			
Tumor necrosis factor alpha	Inflammatory cell activation	x (Garey et al., 2004)			
Total protein	Capillary/epithelial leak	x (Garey et al., 2004)			
(Induced) sputum					
Neutrophils	Inflammation	x (Chalmers et al., 2001; Traves et al., 2002)	x (Swan et al., 1992)		
Eosinophils	Inflammation	x (Chalmers et al., 2001; Dippolito et al., 2001; Petays et al., 2003)			
Eotaxin	Inflammatory cell activation	x (Yamamoto et al., 2003)			
Interleukin-8	Inflammatory cell activation	x (Chalmers et al., 2001; Keatings et al., 1996)	x (Hill et al., 2000)		
Bronchoalveolar lavage (BAL)[b]					
Macrophages	Inflammation	x (Reynolds, 1987)	x (Skold, Hed et al., 1992)		x (Rennard et al., 1990, 1994, 2002[c])
Neutrophils	Inflammation	x (Reynolds, 1987)			x (Rennard et al., 2002[c])
Cytokines	Inflammatory cell activation				
Elastase	Inflammatory cell activation	x (Rennard et al., 1990)			x (Rennard et al., 1990)
Proteins		x (Mio et al., 1997)			
Goblet cell metaplasia[d]	Altered tissue structure	x (Roth et al., 1998; Spurzem et al., 1991)			x (Rennard et al., 2002[c])
2. Functional biomarkers					
Spirometry	Airflow[e]		x (Anthonisen et al., 1994)		
[99m]Tc DTPA clearance	Permeability	x (Kennedy et al., 1984)	x (Minty et al., 1981)		

Table 2. *(Continued.)*

Biomarker	Measurement of	Relation to tobacco product use			
		Difference: users vs. nonusers	Change with cessation	Dose response with use	Change with reduced use
Mucociliary clearance	Mucus secretion and cilia beating	x (Isawa et al., 1984; Pavia et al., 1971)			
3. Directly observed biomarkers Visual inspection of airways	Inflammation	x (Thompson et al., 1989, 1993)			x (Rennard et al., 1990, 1994, 2002[e])

Note. x, relation observed. [a]While primarily relevant for COPD, these markers are also relevant for interstitial lung disease, asthma, and the like. [b]A large number of parameters have been measured in BAL fluid. The ones listed here have been selectively studied in smokers. Many have been studied in smokers in comparison to nonsmokers in studies of specific diseases. No difference was often found, but the limited size of most of the studies precludes definitive conclusion. [c]Changes associated with PREP use. [d]Goblet cells have been assessed in both BAL specimens and in endobronchial biopsy specimens. [e]This is the defining progressive feature of COPD. Improvement following cessation has been demonstrated, but only in patients with COPD.

promises to define hundreds of potential biomarkers, but it will be a challenge to demonstrate their specificity.

As noted above, the cellular and chemical biomarkers assessed in the lung are believed to relate to disease pathogenesis. Most reflect inflammation, as a measure of inflammatory cells, their mediators, or the factors that lead to their recruitment and activation. Because these processes are not specific to cigarette smoke-induced disease, it seems unlikely that any of these markers will be specific to the effects from smoking. By contrast, high levels and patterns of biomarkers seen in smokers may lead to indices with greater specificity.

Biomarkers in addition to chemical and cellular ones also are relevant for nonmalignant lung disease. Physiological measures have been used (Table 2.2 and 2.3). Measures of airflow are well established. Progressive loss of lung function is the defining feature of COPD (NHLB/WHO Workshop Panel, 2003). Improvement in lung function following smoking cessation has been reported, but a large number of subjects were required because of the variance in the airflow measure (Anthonisen et al., 1994). Smoking also is associated with an increase in airway permeability that normalizes rapidly following smoking cessation (Minty, Jordan, & Jones, 1981). Mucociliary clearance is abnormal in smokers (Isawa, Teshima, Hirano, Ebina, & Konno, 1984; Pavia, Thomson, & Pocock, 1971). Other physiological or adaptive lung responses could be explored as biomarkers of cigarette smoke effect.

Imaging methods may also be used to assess biomarkers. Inflammation visualized at the time of bronchoscopy has been used in a variety of studies and has been applied to harm reduction (Thompson et al., 1993). Routine chest radiography is not sensitive enough to detect the abnormalities caused by smoking until they become severe. Thus routine chest radiography is not of much utility to gauge the effect of smoking and is unlikely to be helpful to assess harm reduction. More recent imaging modalities, such as computed tomography (CT) scanning or hyperpolarized gas imaging, show promise for emphysema and assessment of airways; however, their utility in any setting remains to be determined.

Cardiovascular disease biomarkers

Cigarette smoking accelerates atherosclerosis, producing premature atherosclerosis in coronary arteries, the aorta, the carotid and cerebral arteries, and the large arteries in the peripheral circulation (Burns, 2003). Smoking is also associated with an increased risk of acute cardiovascular events, including acute myocardial infarction, sudden death, and stroke (Burns, 2003). Other effects include aggrava-

tion of angina pectoris, intermittent claudication and vasospastic angina, rethrombosis after thrombolysis, and restenosis after coronary bypass surgery or angioplasty (Burns, 2003).

Biomarkers for cardiovascular disease risk can be divided into three categories: (a) Constituents of cigarette smoke that contribute to cardiovascular disease; (b) physiological changes that involve potential mechanisms of cardiovascular disease; and (c) biomarkers of cardiovascular dysfunction and disease. Table 3 shows the biomarkers for potential risk for cardiovascular disease. Although cigarette smoking has been shown to alter a number of cardiovascular biomarkers, as evidenced by comparisons of smokers versus nonsmokers versus ex-smokers, far fewer studies have prospectively examined the reversal of such changes after smoking cessation. More important, no data are available on how changes in smoking-related biomarkers predict future disease risk.

Three constituents of cigarette smoke have received the greatest attention as potential contributors to cardiovascular disease. These are carbon monoxide (measured as exhaled carbon monoxide or blood carboxyhemoglobin), nicotine/cotinine, and oxidant chemicals (Benowitz, 2003). These constituents are widely used as biomarkers of tobacco or tobacco smoke exposures generally; anatabine and anabasine also would fit in this category (Jacob et al., 2002). We are aware of no direct measures of concentrations of oxidizing chemicals in the body, although there are a number of measurements of biological consequences of exposure to oxidizing chemicals, as discussed below. A lesser body of research suggests that PAHs and other constituents of tobacco smoke may contribute to atherogenesis (Benowitz, 2003). Urine concentrations of PAHs, particularly 1-hydroxypyrene, can be measured in smokers (Table 1).

A number of physiological changes involving potential mechanisms of smoking-induced cardiovascular disease have been observed in cigarette smokers compared with nonsmokers. Some of these changes are associated with specific smoke constituents, such as oxidant chemicals, and others are associated with specific cardiovascular disease pathways. The main mechanisms currently believed to be involved include oxidative stress (Burke & Fitzgerald, 2003), hemodynamic effects (Czernin & Waldherr, 2003), endothelial damage or dysfunction or both (Puranik & Celermajer, 2003), enhanced thrombosis (Benowitz, 2003), inflammation (Benowitz, 2003), insulin resistance (Eliasson, 2003), and adverse effects on lipids (Benowitz, 2003).

Cigarette smoke exposes an individual to high concentrations of potentially oxidizing chemicals. Cigarette smoking increases levels of lipid peroxidation products, such as F_2 isoprostanes, in the plasma and urine. Other markers of oxidative stress include higher plasma levels of oxidized low-density lipoprotein (LDL) and oxidized fibrinogen, as well as higher levels of thiobarbituric acid reactive substances in the urine. As a reflection of oxidative stress, plasma levels of antioxidant vitamins such as vitamin E, vitamin C, and beta-carotene are reduced (Burke & Fitzgerald, 2003).

Hemodynamic effects of cigarette smoking may be observed while an individual is smoking. These effects include elevations in heart rate, blood pressure, and cardiac output. Coronary blood flow, as assessed by coronary perfusion studies, may increase or decrease, depending on underlying atherosclerosis and endothelial function (Czernin & Waldherr, 2003).

A number of biomarkers have been proposed to measure endothelial dysfunction, and many of these biomarkers are affected by cigarette smoking. The most widely used functional study is flow-mediated dilation (Puranik & Celermajer, 2003). This test involves the measurement of brachial artery diameter in response to changes in forearm blood flow. The brachial artery is imaged using Doppler ultrasound techniques before and after release of a blood pressure cuff that is inflated over the forearm to occlude arterial blood flow. With the release of the cuff, the increase in blood flow triggers an increase in brachial artery diameter that is mediated by the release of nitric oxide and prostacyclin by endothelial cells. Many researchers have shown impairment of flow-mediated dilation in populations of active and passive smokers, although considerable overlap exists with estimates of these parameters obtained in nonsmokers. Other potential markers of endothelial dysfunction can be measured in the blood, including asymmetric dimethylarginine (ADMA), Von Willebrand factor, tissue plasminogen activator (t-PA), E-selectin, and P-selectin, as well as prostacyclin metabolites in the urine (Cooke, 2000). The selectins are adhesion molecules that are released both by endothelial cells and by platelets (Ley, 2003).

Markers of a hypercoagulable state include increased urine concentrations of thromboxane A2 metabolites. Thromboxane A2 is released when platelets aggregate in vivo, and its metabolites in urine represent a useful noninvasive measure of point of activation (J. Nowak, Murray, Oates, & FitzGerald, 1987). Other relevant biomarkers of a hypercoagulable state include fibrinogen, red blood cell mass, blood viscosity, t-PA, plasminogen activator inhibitor (PAI-1), homocysteine, and P-selectin (Benowitz, 2003).

A number of biomarkers are used to assess inflammatory states (Pearson et al., 2003). These biomarkers include total leukocyte and neutrophil

Table 3. Cardiovascular disease biomarkers.

Biomarker	Measurement of	Relation to tobacco product use			
		Difference: users vs. nonusers	Change with cessation	Dose response with use	Change with reduced use
1. Chemical biomarkers					
Carbon monoxide	Potential chemical toxins	x (SRNT Subcommittee on Biochemical Verification, 2002)	x (SRNT Subcommittee on Biochemical Verification, 2002)	x (Benowitz & Jacob, 1984)	x (Hecht, Murphy et al., 2004)
Nicotine/cotinine	Potential chemical toxins	x (SRNT Subcommittee on Biochemical Verification, 2002)	x (SRNT Subcommittee on Biochemical Verification, 2002)	x (Benowitz & Jacob, 1984)	x (Benowitz et al., 1983)
2. Biomarkers of smoking effects					
Urine F_2- isoprostanes	Oxidative stress	x (J. D. Morrow et al., 1995)	x (Pilz et al., 2000)		
Thiobarbituric acid reactive substances	Oxidative stress				
Serum levels of vitamin C	Oxidative stress	x (Lykkesfeldt et al., 2000)			
Heart rate	Hemodynamic effects	x (Benowitz et al., 1984)	x (Benowitz et al., 1984)		
Blood pressure	Hemodynamic effects	x (Benowitz et al., 2002)	x (Benowitz et al., 2002)		
Nuclear coronary perfusion studies	Hemodynamic effects	x (Czernin & Waldherr, 2003)	–	–	x (Mahmarian et al., 1997)
Flow-mediated dilation	Endothelial function	x (Czernin & Waldherr, 2003)	x (Czernin & Waldherr, 2003)	x (Czernin & Waldherr, 2003)	
Circulating endothelial precursor cells	Endothelial function	x (Kondo et al., 2004)	x (Kondo et al., 2004)	x (Kondo et al., 2004)	
von Willebrand factor	Endothelial function	x (Blann et al., 1997)	x (Blann et al., 1997)		
P-selectin	Endothelial function	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)		
E-selectin	Endothelial function	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)		
Urine thrombaxane A_2-metabolite	Hypercoagulable state	x (J. Nowak et al., 1987)	x (Saareks et al., 2001)		
Fibrinogen	Hypercoagulable state	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)	
Red blood cell mass	Hypercoagulable state	x (Blann et al., 1997)	x (Blann et al., 1997)		
Homocysteine	Hypercoagulable state	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)	
Tissue plasminogen activator	Hypercoagulable state	x (Simpson et al., 1997)			
White blood cell count	Inflammation	x (Jensen et al., 1998)	x (Jensen et al., 1998)	x (Jensen et al., 1998)	
C-reactive protein	Inflammation	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)	x (Bazzano et al., 2003)	
Interleukin-6	Inflammation	x (Bermudez et al., 2002)	x (Bermudez et al., 2002)		
Soluble intracellular adhesion molecule-1 (sICAM-1)	Inflammation	x (Scott et al., 2000)	x (Scott et al., 2000)	x (Scott et al., 2000)	
Hemoglobin A_1C	Insulin resistance	x (Sargeant et al., 2001)			
Insulin/glucose ratio	Insulin resistance	x (Zavaroni et al., 1994)			
Glucose-clamping studies	Insulin resistance	x (Eliasson et al., 1997)	x (Eliasson et al., 1997)	x (Eliasson et al., 1997)	
HDL cholesterol	Lipid marker	x (Stubbe et al., 1982)	x (Stubbe et al., 1982)		
Oxidized LDL cholesterol	Oxidative stress/lipid marker	x (Panagiotakos et al., 2004)	x (Panagiotakos et al., 2004)		
Serum triglycerides	Lipid marker	x (Axelsen et al., 1995)			
Carotid and femoral artery intima-media thickness	Atherosclerosis	x (Wallenfeldt et al., 2001)			

Note. x, relation observed. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

counts, C-reactive protein, fibrinogen, and interleukin-6. In addition, a number of cell surface adhesion molecules are increased in inflammatory states, including soluble intracellular adhesion molecule (sICAM), soluble vascular cell adhesion molecule (sVCAM-1), and monocyte chemoattractant protein-1 (MCP-1).

A number of markers are useful to assess insulin resistance (Eliasson, 2003). Fasting and 2-hr postprandial plasma glucose may be elevated in the presence of insulin resistance. Hemoglobin A_1C levels, reflecting plasma glucose throughout the day, would be expected to be elevated if hyperglycemia is present. The ratio of insulin to glucose after glucose load is useful as an index of insulin sensitivity. The most definitive studies are glucose-clamping studies, in which insulin levels are measured when a constant concentration of glucose is present or vice versa.

Several standard markers assess lipid levels, which may be altered in cigarette smokers. These include high-density lipoprotein (HDL) cholesterol, LDL cholesterol, total cholesterol/HDL ratio, and serum triglycerides (Table 3).

Functional studies are useful in assessing the presence of cardiovascular dysfunction or disease. These assessments include nuclear coronary perfusion studies, without and with exercise. Such studies indicate that cigarette smoking reduces cardiac perfusion in patients with coronary disease (Czernin & Waldherr, 2003). Endothelial function reserve can be assessed by studying flow-mediated dilation, as described previously. An assessment of vascular disease can be achieved by measuring carotid and femoral artery intima-media thickness (de Groot et al., 2004) by ultrasonography, which provides a direct measurement of early atherosclerotic changes of blood vessels.

We have mentioned a number of cardiovascular biomarkers that might be used to assess effects of cigarette smoking and that are expected to increase the risk of cardiovascular disease. Many biomarkers are not reflective of causal pathways related to the development of cardiovascular disease but rather reflect pathophysiological effects of cigarette smoke constituents. Another problem is that many biomarkers are influenced by processes and risk factors independent of cigarette smoking. Many of the same abnormalities produced by smoking also are produced by diabetes, hypercholesterolemia, and hypertension. Thus it is unclear which biomarkers are most specific to cigarette smoking. Also unclear is which biomarkers best predict the risk of cardiovascular diseases attributable to cigarette smoke. Additionally, with a given biomarker profile, marked differences exist in individual susceptibility to cardiovascular disease.

Improved biomarkers for cardiovascular disease could potentially be developed using advances in high throughput genomics and by examining the relationships of gene polymorphisms or alterations in protein expression or activity to smoking-induced disease (Zhang, Day, & Ye, 2001). Emerging genomic and proteomic technologies may cast light on the signaling pathways activated by smoking and constituents of smoke that culminate in cardiovascular dysfunction. Such approaches may contribute to our understanding of interindividual differences in susceptibility to cardiovascular complications of smoking.

A number of studies of clinical genetics have examined differences in susceptibility to smoking-induced cardiovascular disease as a function of different genetic variants (Wang, Raveendran, & Wang, 2003). Such studies, combined with genomic and proteomic approaches, may provide both mechanistic information on pathogenesis and—in combination with other biomarkers—better prediction of cardiovascular risk in smokers.

Prenatal tobacco exposure and fetal toxicity biomarkers

Cigarette smoking adversely affects many aspects of reproduction including an increased risk of delayed conception and infertility, ectopic pregnancy, spontaneous abortion, preterm premature rupture of membranes, placental complications, premature delivery, low-birth-weight infants, abnormalities in fetal lung development, stillbirth, and neonatal and perinatal mortality (Stratton et al., 2001; U.S. Department of Health & Human Services [USDHHS], 2001). Moreover, prenatal tobacco exposure is associated with an increased risk of sudden infant death syndrome (Milerad, Vege, Opdal, & Rognum, 1998), childhood cognitive and behavioral abnormalities (Fried & Watkinson, 1988, 2001; Linnet et al., 2003), childhood asthma (Stratton et al., 2001), nicotine dependence in the offspring (Kandel, Wu, & Davies, 1994), and childhood obesity (Ong, Preece, Emmett, Ahmed, & Dunger, 2002; Wideroe, Vik, Jacobsen, & Bakketeig, 2003). Some evidence indicates that prenatal tobacco exposure increases the risk of childhood cancers, but the results are not consistent (Bofetta, Tredaniel, & Greco, 2000).

Smoking may cause fetal toxicity by direct toxic effects of a number of chemicals, fetal hypoxia, inappropriately timed and excessive stimulation of nicotinic receptors, oxidative stress, DNA damage, inflammation, alterations in endothelial function, hemodynamic effects or reduced placental blood flow, placental alterations and function, or hormonal changes. A detailed review of the mechanisms by

which smoking causes fetal harm can be found elsewhere (Dempsey & Benowitz, 2001). This section addresses biomarkers of fetal toxicity exclusively from prenatal exposure via maternal smoking. Three categories of outcomes that show the most promise as biomarkers for fetal toxicity that may be sensitive to tobacco reduction include birth weight, lung function, and neurotoxicity in the offspring (Table 4.1). Other biomarkers potentially associated with these outcomes are listed in Table 4.2.

Birth weight

Many studies show a dose-response relationship between measures of tobacco exposure and infant birth weight (USDHHS, 2001; Walsh, 1994). The average weight difference in the offspring of smokers compared with nonsmokers is about 250 g (USDHHS, 2001). Maternal smoking doubles the risk of delivering a low-birth-weight infant (i.e., <2,500 g; Li, Windsor, Perkins, Goldenberg, & Lowe, 1993; Sexton & Hebel, 1984; USDHHS, 2001; Walsh, 1994). Moreover, evidence indicates that smoking reduction improves birth weight. A 60 mg/ml reduction in maternal cotinine levels during pregnancy results in a 92-g increase in birth weight (Sexton & Hebel, 1984). A reduction in nine cigarettes smoked per day or more than 8 ppm exhaled carbon monoxide is associated with a 100-g increase in birth weight (Li et al., 1993). However, numerous confounders may influence the association between smoking and birth weight (Walsh, 1994). Moreover, the association between maternal smoking and birth weight may not be linear, and large reductions in exposure may be necessary to produce measurable effects on birth weight.

Although birth weight or birth weight corrected for gestational age are the best indicators of fetal growth, maternal smoking also has measurable effects on crown-heel length, abdominal circumference, and head circumference (Cliver et al., 1995; Goldenberg et al., 1993; Wang, Tager, Van Vunakis, Speizer, & Hanrahan, 1997). Fetal growth can be assessed by serial ultrasound measurements, although the effects of smoking on growth are not detected by this method until late in the third trimester (Goldenberg et al., 1993). One disadvantage of using birth weight or ultrasound measurements as biomarkers of fetal growth is that they reflect tobacco exposure primarily in the latter half of pregnancy. Quitting smoking at 16 weeks' (MacArthur & Knox, 1988) and even as late as 30 weeks' gestation produces offspring of similar weight compared with the offspring of nonsmokers (Ahlsten, Cnattingius, & Lindmark, 1993).

Several mechanisms may lead to cigarette-related reduced birth weight and may serve as biomarkers associated with low weight. These biomarkers are indicators of the extent of oxygen transport, vasoconstriction, oxidative stress, endothelial dysfunction, blood flow, and nutrient exchange. However, no single biomarker may be sufficiently predictive of low birth weight.

Carbon monoxide in cigarette smoke binds to fetal hemoglobin and reduces the availability of oxygen to the fetus (Harrison & Robinson, 1981; Secker-Walker, Vacek, Flynn, & Mead, 1997a, 1997b), which could adversely affect fetal growth. Indeed, animal studies show that pregnant rats exposed to carbon monoxide have measurable reductions in fetal growth (Lynch & Bruce, 1989). Nicotine could theoretically affect fetal growth through vasoconstrictive effects on the placenta or through direct toxic effects, although an effect of nicotine on fetal growth has not been demonstrated in human research (Oncken et al., 1996; Wisborg, Henricksen, Jespersen, & Secher, 2000). Nicotine as well as oxidant gases also could impair endothelial function (Ahlsten, Ewald, & Tuvemo, 1986). Alterations in endothelial function (i.e., reduced cellular fibronectin and increased intracellular adhesion molecule) have been demonstrated in pregnant smokers (Lain, Wilson, Crombleholme, Ness, & Roberts, 2003). Endothelial dysfunction in the placental circulation affects release of vasodilating compounds such as prostacyclin (PGI_2) and nitric oxide, which are important in regulating the placental blood flow necessary for oxygen and nutrient exchange (Obwegeser, Oguogho, Ulm, Berghammer, & Sinzinger, 1999). Reduced release of prostacyclin (Ahlsten et al., 1986; Dadak, Kefalides, Sinzinger, & Weber, 1982; Obwegeser et al., 1999; Ulm, Plockinger, Pirich, Gryglewski, & Sinzinger, 1995) and nitric oxide (Obwegeser et al., 1999; Ozerol, Ozerol, Gokdeniz, Temel, & Akyol, 2004; Ulm et al., 1995) have been observed in umbilical cord blood in smokers versus nonsmokers.

Although smoking is commonly theorized to reduce uteroplacental blood flow, Doppler studies have demonstrated conflicting results (Bruner & Forouzan, 1991; Castro, Allen, Ogunyemi, Roll, & Platt, 1993; Lindblad, Marsal, & Andersson, 1988; R. J. Morrow, Ritchie, & Bull, 1988; Oncken et al., 1996), which may be related to the limitation of the measure (Castro et al., 1993). Radionucleotide studies, however, suggest that placental perfusion is decreased in smokers versus nonsmokers (Philipp, Pateisky, & Endler, 1984). Placental pathology studies also suggest hypoperfusion of the placenta from the maternal circulation (Naeye, 1978).

Other hemodynamic effects occur with smoking cessation; however, whether they influence fetal

Table 4. Prenatal tobacco exposure and fetal toxicity biomarkers.

		Relation to tobacco product use			
Biomarker	Measurement of	Difference: users vs. nonusers	Change with cessation	Dose response	Change with reduced use
1. Directly observed biomarkers					
Birth weight	Outcome: fetal growth	x (USDHHS, 2001; Walsh, 1994)	x (Li et al., 1993; Sexton & Hebel, 1984)	x (USDHHS, 2001; Walsh, 1994)	x (Li et al., 1993; Secker-Walker et al., 1997b)
Pulmonary function tests	Outcome: lung function	x (Gilliland et al., 2003)		x (Stick et al., 1996)	
Auditory habituation/orientation in offspring	Outcome: neurotoxicity	x (Fried & Makin, 1987; Picone et al., 1982; Saxton, 1978)			
Neurocognitive impairments in offspring	Outcome: neurotoxicity	x (Fried & Watkinson, 2001; Fried et al., 1998; Fried et al., 1997; McCartney et al., 1994; Naeye & Peters, 1984)	x (Sexton et al., 1990)	x (Fried et al., 1997, 1998)	
2. Chemical and cellular biomarkers					
Maternal exhaled carbon monoxide	Potential chemical toxin	x (Christensen et al., 2004)	x (Secker-Walker et al., 1997a, 1997b)	x (Secker-Walker et al., 1997a, 1997b)	x (Secker-Walker et al., 1997a, 1997b)
Maternal carboxyhemoglobin, carboxyhemoglobin in cord blood	Potential chemical toxin	x (Harrison & Robinson, 1981)		x (Harrison & Robinson, 1981)	
Increased fetal hemoglobin levels	Carboxyhemoglobin exposure	x (Naeye & Peters, 1984)		x (Naeye & Peters, 1984)	
NNAL in amniotic fluid, and NNAL and NNAL-Glucs in infant urine after delivery	Carcinogen (NNK) uptake	x (Lackmann et al., 1999; Milunsky et al., 2000)			
PAH DNA adducts in white blood cells	Carcinogen uptake and metabolic activation	x (Perera et al., 1999)			
4-Aminobiphenyl Hb adducts (umbilical vein)	Carcinogen uptake and metabolic activation	x (Coghlin et al., 1991; Myers et al., 1996)		x (Coghlin et al., 1991; Myers et al., 1996)	
Maternal cotinine (urine, saliva, plasma)	Potential chemical toxin	x (Oncken et al., 2003)	x (Li et al., 1993)	x (Wang et al., 1997)	x (Li et al., 1993)
Cotinine in meconium	Potential chemical toxin	x (Derauf et al., 2003; Ostrea et al., 1994)		x (Ostrea et al., 1994)	
Nicotine and cotinine in infant hair after delivery	Potential chemical toxin	x (Eliopoulos et al., 1994)			
F_2 isoprostanes in cord blood	Oxidative stress	x (Obwegeser et al., 1999)			
Amniotic fluid and plasma vitamin C	Oxidative stress	x (Barrett et al., 1991; Cogswell et al., 2003[a])			
Thiocyanate in maternal saliva and in maternal/umbilical cord blood	Hydrogen cyanide uptake	x (McMahon et al., 1997)	x (Sexton & Hebel, 1984)	x (McMahon et al., 1997)	
Cadmium in placenta and maternal and cord blood	Metal uptake	x (Falcon et al., 2002; Larsen et al., 2002; Piasek et al., 2001)		x (Larsen et al., 2002)	
Maternal: intracellular adhesion molecule 1 (ICAM)	Endothelial function	x (Lain et al., 2003)	x (Lain et al., 2003)		
cFN (cellular fibronectin)	Endothelial function	x (Lain et al., 2003)			
Fetal: prostacyclin, L-arginine, L-citrulline in umbilical arteries and veins	Endothelial function	x (Ahlsten et al., 1986; Dadak et al., 1982; Obwegeser et al., 1999; Ulm et al., 1995)			
Total serum nitrite	Endothelial function	x (Ozerol et al., 2004)			
Placental pathology	Tissue biomarkers	x (Naeye, 1978)		x (Naeye, 1978)	

Table 4. *(Continued.)*

Biomarker	Measurement of	Relation to tobacco product use			
		Difference: users vs. nonusers	Change with cessation	Dose response	Change with reduced use
Serial ultrasound for fetal growth	Radiologic biomarkers	x (Goldenberg et al., 1993)			
Placental flood flow measurement (radioisotopes)	Radiologic biomarkers	x (Philipp et al., 1984)			
Homocysteine	Hypercoagulable state	x (Ozerol et al., 2004)			
Folate vitamins B12 and B6 concentrations	Other biomarkers	x (Cogswell et al., 2003[a]; Ozerol et al., 2004)			
Fetal catecholamine levels	Other biomarkers	x (Oncken et al., 2003)			
Maternal estrogen levels	Hormonal concentrations	x (Kaijser et al., 2000; Petridou et al., 1990)			

Note. x, relation observed [a]review article, most studies show relationship.

growth is not known. For example, morning baseline fetal heart rate decreases after 1 week of maternal smoking cessation with nicotine patch (Ogburn et al., 1999), presumably reflecting reduced fetal sympathetic nervous system activation. Fetal breathing movements and fetal heart rate variability are acutely altered by smoking. The mechanism may be either fetal hypoxia or direct effects of nicotine (Oncken, Hardardottir, & Smeltzer, 1998).

Hydrogen cyanide (HCN) in tobacco smoke could contribute to low birth weight by interfering with fetal tissue oxygen utilization (Walsh, 1994) and decreasing levels of vitamin B12 (Cogswell, Weisberg, & Spong, 2003; Walsh, 1994). Vitamin B6 and folate concentrations also are decreased, and homocysteine concentrations are increased in pregnant smokers, which could affect fetal growth (Cogswell et al., 2003; Ozerol et al., 2004). Plasma homocysteine levels are elevated in folate deficiency as well as in vitamin B12 deficiency because these vitamins function as coenzymes in the metabolism of total homocysteine. It is not known whether vitamin deficiencies and increased homocysteine concentrations observed in some studies of pregnant smokers affect growth independently or if the effects are additive.

Other chemicals in tobacco smoke such as cadmium and lead could have deleterious effects on fetal growth (Falcon, Vinas, Osuna, & Luna, 2002). Cadmium levels are increased in the placentas of smokers compared with nonsmokers, which may affect placental function (Falcon et al., 2002; Piasek, Blanusa, Kostial, & Laskey, 2001). Studies in animals show that cadmium accumulation in the placenta impairs transfer of essential minerals and nutrients for fetal growth (Piasek et al., 2001). Stereologic examination of placentas of smokers shows reduced volume, surface area, and length for villous capillaries possibly as a result of cadmium exposure (Larsen, Clausen, & Jonsson, 2002). The authors suggest that cadmium has a direct toxic effect on placental tissue, causing a decrease in the volume density of the placental vasculature necessary for oxygen and nutrient exchange.

Cigarette smoking may affect birth weight by reducing estrogen levels. Although the mechanisms of the relationship among smoking, estrogen levels, and birth weight are not known, estrogens are established growth factors in many biological systems (Kaijser, Granath, Jacobsen, Cnattingius, & Ekbom, 2000; Petridou, Panagiotopoulou, Katsouyanni, Spanos, & Trichopoulos, 1990). Pregnant smokers have 10%–20% lower estrogen levels compared with nonsmokers (Kaijser et al., 2000; Petridou et al., 1990), and the placental progesterone concentrations also are reduced compared with nonsmokers (Piasek et al., 2001). Whether

the effects of smoking on maternal estrogen are independent of the effects on placental progesterone are unknown because studies have focused on one or the other.

Carcinogens have been hypothesized to contribute to low birth weight via DNA damage or oxidative stress (Wang et al., 2002). Metabolites and adducts of several tobacco smoke carcinogens are detectable in tissues of women who smoked during pregnancy and in tissues of fetuses and offspring of women who smoked during pregnancy. Levels of metabolites of NNK (NNAL and NNAL-Glucs) are higher in the amniotic fluid of smokers versus nonsmokers (Milunsky, Carmella, Ye, & Hecht, 2000) and in first urine from infants of mothers who smoke (Lackmann et al., 1999). 4-Aminobiphenyl-Hb adduct levels are higher in maternal and fetal blood in smokers versus nonsmokers at the time of delivery (Coghlin et al., 1991; Myers et al., 1996). PAH DNA adduct levels in maternal white blood cells are higher in smokers versus nonsmokers (Perera, Jedrychowski, Rauh, & Whyatt, 1999). In one study of 741 pregnant women (Wang et al., 2002), those with polymorphisms of CYP1A1 or GSTT1 (phase 1 and phase 2 enzymes of xenobiotic metabolism that may affect activation or elimination of carcinogens such as PAHs) had significantly lower-birth-weight infants if they smoked during pregnancy. Genetic polymorphisms had no effect on birth weight in the offspring of nonsmokers, suggesting a gene-environment interaction (Wang et al., 2002).

Lung function

Because exposures during pregnancy are likely to be similar to early childhood exposures, available epidemiological data relating specifically to fetal exposure are limited. Nevertheless, several studies suggest that fetal exposure to smoke could contribute to the development of COPD and asthma. Prenatal exposure to smoke increases rates of respiratory tract infection and otitis media. Additionally, lung function is reduced (Dezateux, Stocks, Wade, Dundas, & Fletcher, 2001; Gilliland et al., 2000; Stick, Burton, Gurrin, Sly, & Le Sour, 1996) and airway resistance increased (Kooi, Vrijlandt, Boezen, & Duiverman, 2004), an effect that may be related to impaired lung development. Reduced lung function in children exposed to maternal smoking during pregnancy also is observed into adulthood (Svanes et al., 2004). A reduction in alveolar attachment in infants exposed to smoke in utero (Elliot, Carroll, James, & Robinson, 2003) may both contribute to the development of COPD and predispose to wheezing (Saetta et al., 1985). Consistent with an effect on lung development is an increased risk for bronchopulmonary dysplasia in children born to smoking mothers (Antonucci, Contu, Porcella, Atzeni, & Chiappe, 2004). Asthma is increased in children whose mothers smoked during pregnancy (Ehrlich et al., 1996), and even passive smoke exposures of pregnant women have been reported to have an effect (Barber, Mussin, & Taylor, 1996).

As mentioned earlier, maternal cigarette smoking is also a cause of low birth weight (USDHHS, 2004). This is a separate risk factor for asthma (DiFranza, Aligne, & Weitzman, 2004) and, while controversial, has been suggested to be a risk factor for the development of COPD (Barker et al., 1991). Lung function is maximal in young adulthood, and a low birth weight is associated with lower maximally attained function (Barker et al., 1991). A lower maximally attained lung function in turn has been reported to increase risk for COPD (Sherrill, Lebowitz, Knudson, & Burrows, 1991; Tager, Segal, Speizer, & Weiss, 1988).

Neurotoxicity

Age-appropriate neurocognitive and developmental outcomes may also serve as biomarkers of potential harm for prenatal tobacco smoke exposure. Although it is debatable whether neurocognitive and developmental tests should be labeled ''outcomes'' or ''biomarkers,'' the fetal toxicity workgroup considers them to be both. The effects of prenatal tobacco exposure on neurocognitive and developmental tests are subtle and do not necessarily indicate a disease (i.e., attention deficit hyperactive disorder [ADHD], mental retardation) or even impairment in school performance. Because the effects of prenatal tobacco exposure on neurocognitive and developmental tests can be viewed as a continuum, and because these outcomes are reliable (although not necessarily robust or dramatic) across studies, we feel it is appropriate to view developmental, neurocognitive, and behavioral tests in the offspring of mothers who smoke as biomarkers of potential harm.

Newborns exposed to tobacco prenatally exhibit impaired auditory habituation (an indication of nervous system integrity) (Fried & Makin, 1987; Picone, Allen, Olsen, & Ferris, 1982; Saxton, 1978) and increased tremors, hypertonicity, and nervous system excitation compared with offspring of nonsmokers (Fried, Watkinson, Dillon, & Dulberg, 1987). Maternal cigarette smoking during pregnancy is associated with decreased verbal comprehension in children aged 1–2 years (Fried & Watkinson, 1988). Longitudinal studies show a dose-dependent association between prenatal tobacco exposure and auditory-related cognitive deficits in children and adolescents (Fried & Watkinson, 2001; Fried, Watkinson, & Gray, 1998; Fried, Watkinson, &

Siegel, 1997; McCartney, Fried, & Watkinson, 1994; Naeye & Peters, 1984). Additionally, cohort studies suggest a dose-dependent relationship between maternal smoking and attention (freedom from distractibility index) in the offspring (Fried & Watkinson, 2001). Because neurocognitive and behavioral outcomes are dose related to maternal smoking, and evidence indicates that smoking cessation during pregnancy improves neurocognitive outcomes (Sexton, Fox, & Hebel, 1990), age-appropriate developmental and cognitive tests assessing overall IQ, auditory processing, and attention are important outcome biomarkers to consider in PREP studies.

Carbon monoxide, nicotine, HCN, lead, arsenic, cadmium, and potential carcinogens such as PAHs have been theorized to contribute to fetal and childhood neurotoxicity associated with tobacco smoke. Carbon monoxide and HCN interfere with oxygen delivery and tissue utilization in the developing brain, leading to hypoxic cell injury (Mactutus, 1989). The fetal brain is sensitive to hypoxic cell injury because of high relative oxygen consumption and low concentrations of natural antioxidant molecules (Hamrick & Ferriero, 2003). Nicotine, which is structurally similar to acetylcholine, inhibits neural cell replication and differentiation, leading to cell loss, disruption of brain architecture, and miswiring of neural connections in animal models. The mechanism appears to be inappropriately timed and excessive stimulation of nicotinic cholinergic receptors involved in trophic effects of acetylcholine in the developing brain (Slotkin, Lappi, McCook, Lorber, & Seidler, 1995). One study reported a decreased catecholamine response to delivery in the offspring of smokers versus nonsmokers (Oncken et al., 2003). The third trimester in humans is when ingrowth of thalamocortical axon terminals, transient expression of nicotinic receptors in the auditory cortex of nicotinic receptors, and the onset of hearing occur. Findings of decreased auditory habituation in the offspring of mothers who smoke, and of long-term auditory-related cognitive deficits in children exposed prenatally to tobacco, suggest that the fetal brain is vulnerable to exogenous nicotine (Metherate & Hsieh, 2003). Lead, arsenic, and cadmium could damage developing neurons through multiple mechanisms; indeed, these metals share the ability to elicit oxidative stress and consequent damage in nucleic acids and membrane lipids (Olanow & Arendash, 1984). Additionally, PAHs have been implicated in decreased head circumference and potentially decreased long-term intelligence by direct DNA damage in utero (Perera et al., 1999).

Potential mechanisms by which prenatal nicotine exposure could contribute to neurobehavioral abnormalities similar to ADHD include modulation of the dopaminergic system and an increased number of nicotinic receptors (Linnet et al., 2003). Elevated fetal hemoglobin concentrations at birth are associated with behavioral abnormalities in children aged 7 years who were born to smokers, suggesting that fetal hypoxemia may be the mechanism by which maternal smoking contributes to the genesis of behavioral abnormalities (Naeye & Peters, 1984).

Studies of PREPs and neurotoxicity should include biomarkers of tobacco exposure and assessment of neurocognitive and behavioral outcomes previously discussed. Markers of oxidative stress in maternal circulation throughout pregnancy as well as in the cord blood at the time of delivery also may be informative. Markers of tobacco exposure in the mother (as a surrogate measure of fetal exposure) could include exhaled carbon monoxide, thiocyanate, cotinine, and carcinogen exposure throughout pregnancy. Overall nicotine exposure can be measured by cotinine. Because nicotine has been implicated in the neurotoxicity of tobacco, measurements of long-term fetal nicotine exposure (i.e., hair analyses or meconium) should be considered in neurological studies where nicotine replacement therapy is used as a PREP.

In summary, some biomarkers (particularly health outcome biomarkers) are sensitive to tobacco exposure in pregnancy. However, many biomarkers are not specific for tobacco-related fetal toxicity. Moreover, research is needed to identify biomarkers of injury or potential harm that can be predictive of various adverse pregnancy and infant outcomes.

Conclusions

Several biomarkers show sufficient sensitivity to changes in smoking status to suggest that they may be useful to assess constituent exposure with PREP use in a research setting. Table 5 lists current biomarkers that show differences between smokers and nonsmokers, change with cessation, and exhibit a dose-response relationship or that respond to reductions in cigarette consumption. The biomarker, 1-HOP, was excluded from this list because it does not exhibit a clear dose-response relationship with numer of cigarettes smoked (Joseph et al., 2005). This table by no means describes biomarkers that can be used to assess disease risk for PREPs. The limited number of biomarkers listed in the table highlights the need for more systematic research to determine biomarkers that are reproducible, that show a dose-response relationship to exposure to tobacco and cigarette smoke toxins, that reflect the spectrum of tobacco-related disease states and mechanisms, and that are predictive of diseases (Hatsukami, Hecht, Hennrikus, Joseph, & Pentel, 2003; Hatsukami et al., 2002; Shields, 2002; Stratton et al., 2001). The

Table 5. Panel of biomarkers.

Biomarkers	Measurement of
Cancer	
NNAL and NNAL-Glucs in urine	Carcinogen (NNK) uptake[b]
3-Aminobiphenyl, 4 aminobiphenyl, and other aromatic amine-Hb adducts	Carcinogen (aromatic amines) uptake plus metabolic activation[c]
Urine mutagenicity	Mutagen uptake[d]
Sister chromatid exchange in peripheral lymphocytes	DNA damage[c]
Nonmalignant lung disease	
Macrophages	Inflammation[d]
Cardiovascular disease	
Carbon monoxide[a]	Chemical uptake[b]
Nicotine/cotinine[a]	Chemical uptake and metabolism[b]
Flow-mediated dilation	Endothelial function[d]
Circulating endothelial precursor cells	Endothelial function[d]
Fibrinogen	Hypercoagulable state[d]
Homocysteine	Hypercoagulable state[d]
White blood cell count	Inflammation[d]
C-reactive protein	Inflammation[d]
sICAM1	Inflammation[d]
Glucose-clamping studies	Insulin resistance[d]
Fetal toxicity	
Birth weight	Outcome[e]
Neurocognitive impairments in offspring	Outcome[e]
Maternal exhaled carbon monoxide	Chemical uptake[b]
Maternal cotinine	Chemical uptake and metabolism[b]
Maternal thiocyanate	Chemical uptake and metabolism[b]

Note. [a]Should be included in all studies as general measures of tobacco constituent uptake. [b]Biomarker for exposure. [c]Biomarker for toxicity including biologically effective dose. [d]Biomarker for injury or potential harm. [e]Health outcome.

challenges associated with assessing harmful effects of PREPs include the potential introduction of new, unknown toxicants; the contribution of new toxicants to the inherent toxicity of tobacco and tobacco smoke; and a need for greater understanding of specific tobacco-related mechanisms associated with pathogenesis. We are also limited in understanding the intra- and interindividual differences in physiology, the complex physiological interactions, and the interactions between susceptibility to disease and the effects from tobacco. Developing a knowledge base in these areas will require systematic and comprehensive research across a multitude of disciplines. Investment in this area is of utmost importance because biomarkers form the basis of evaluating PREPs and, for that matter, tobacco products in general.

Acknowledgments

The following individuals comprised the members of the Biomarkers Workgroup: (a) Cancer-related biomarkers—Steven Belinsky, Peter Shields, and Steven Tannenbaum; (b) nonmalignant pulmonary disease biomarkers—Mark Frampton and David Riley; (c) cardiovascular disease biomarkers—Anne Burke and John Cooke; and (d) prenatal tobacco exposure and fetal toxicity biomarkers—Peter Fried and Theodore Slotkin. The authors thank David Burns, Jonathan Samet, and George Hammons for their valuable comments on the manuscript. This conference was funded by the National Cancer Institute, the National Institute on Drug Abuse, and the National Institute on Alcohol Abuse and Alcoholism. Support for efforts of individual authors comes from grants DA013333 (Hatsukami and Hecht), DA0227 (Benowitz), and DA15167 (Oncken) and from the Larson Endowment, University of Nebraska Medical Center (Rennard).

References

Agius, R. M., Rutman, A., Knight, R. K., & Cole, P. J. (1986). Human pulmonary alveolar macrophages with smokers' inclusions: Their relation to the cessation of cigarette smoking. *British Journal of Experimental Pathology*, *67*, 407–413.

Ahlsten, G., Cnattingius, S., & Lindmark, G. (1993). Cessation of smoking during pregnancy improves foetal growth and reduces infant morbidity in the neonatal period. A population-based prospective study. *Acta Paediatrica*, *82*, 177–181.

Ahlsten, G., Ewald, U., & Tuvemo, T. (1986). Maternal smoking reduces prostacyclin formation in human umbilical arteries. A study on strictly selected pregnancies. *Acta Obstetrricia et Gynecologica Scandinavica*, *65*, 645–649.

Anthonisen, N., Connett, J., Kiley, J., Altose, M., Bailey, W., Buist, A., Conway, W. A., Jr., Enright, P. L., Kanner, R. E., O'Hara, P., Owens, G. R., Scanlon, P. D., Tashkin, D. P., & Wise, R. A. (1994). Effects of smoking intervention and the use of an inhaled anticholinergic bronchodilator on the rate of decline of FEV1: The lung health study. *The Journal of the American Medical Association*, *272*, 1497–1505.

Antonucci, R., Contu, P., Porcella, A., Atzeni, C., & Chiappe, S. (2004). Intrauterine smoke exposure: A new risk factor for bronchopulmonary dysplasia? *Journal of Perinatal Medicine*, *32*, 272–277.

Atawodi, S. E., Lea, S., Nyberg, F., Mukeria, A., Constantinescu, V., Ahrens, W., Brueske-Hohlfeld, I., Fortes, C., Boffetta, P., & Friesen, M. D. (1998). 4-Hydroxy-1-(3-pyridyl)-1-butanone-hemoglobin adducts as biomarkers of exposure to tobacco smoke: Validation of a method to be used in multicenter studies. *Cancer Epidemiology, Biomarkers, & Prevention*, *7*, 817–821.

Axelsen, M., Eliasson, B., Joheim, E., Lenner, R. A., Taskinen, M. R., & Smith, U. (1995). Lipid intolerance in smokers. *Journal of Internal Medicine*, *237*, 449–455.

Balint, B., Donnelly, L. E., Hanazawa, T., Kharitonov, S. A., & Barnes, P. J. (2001). Increased nitric oxide metabolites in exhaled breath condensate after exposure to tobacco smoke. *Thorax*, *56*, 456–461.

Barale, R., Chelotti, L., Davini, T., Del Ry, S., Andreassi, M. G., Ballardin, M., Bulleri, M., He, J., Baldacci, S., Di Pede, F., Gemignani, F., & Landi, S. (1998). Sister chromatid exchange and micronucleus frequency in human lymphocytes of 1,650 subjects in an Italian population: II. Contribution of sex, age, and lifestyle. *Environmental and Molecular Mutagenesis*, *31*, 228–242.

Barber, K., Mussin, E., & Taylor, D. K. (1996). Fetal exposure to involuntary maternal smoking and childhood respiratory disease. *Annals of Allergy, Asthma & Immunology*, *76*, 427–430.

Barker, D. J. P., Godfrey, K. M., Fall, C. H. D., Osmond, C., Winter, P. D., & Shaheen, S. O. (1991). Relation of birthweight and childhood respiratory infection to adult lung function and death from chronic obstructive airways disease. *British Medical Journal*, *303*, 671–675.

Barrett, B., Gunter, E., Jenkins, J., & Wang, M. (1991). Ascorbic acid concentration in amniotic fluid in late pregnancy. *Biology of the Neonate*, *60*, 333–335.

Bazzano, L. A., He, J., Muntner, P., Vupputuri, S., & Whelton, P. K. (2003). Relationship between cigarette smoking and novel risk factors for cardiovascular disease in the United States. *Annals of Internal Medicine*, *138*, 891–897.

Benowitz, N. L. (2003). Cigarette smoking and cardiovascular disease: Pathophysiology and implications for treatment. *Progress in Cardiovascular Disease*, *46*, 91–111.

Benowitz, N. L., Hall, S. M., Herning, R. I., Jacob, P., 3rd., Jones, R. T., & Osman, A. L. (1983). Smokers of low-yield cigarettes do not consume less nicotine. *The New England Journal of Medicine*, *309*, 139–142.

Benowitz, N. L., Hansson, A., & Jacob, P., 3rd. (2002). Cardiovascular effects of nasal and transdermal nicotine and cigarette smoking. *Hypertension*, *39*, 1107–1112.

Benowitz, N. L., Jacob, P., 3rd., Kozlowski, L. T., & Yu, L. (1986). Influence of smoking fewer cigarettes on exposure to tar, nicotine, and carbon monoxide. *The New England Journal of Medicine*, *315*, 1310–1313.

Benowitz, N. L., & Jacob, P. I. (1984). Daily intake of nicotine during cigarette smoking. *Clinical Pharmacology and Therapeutics*, *35*, 499–504.

Benowitz, N. L., Kuyt, F., & Jacob, P., 3rd. (1984). Influence of nicotine on cardiovascular and hormonal effects of cigarette smoking. *Clinical Pharmacology and Therapeutics*, *36*, 74–81.

Bergmark, E. (1997). Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chemical Research in Toxicology*, *10*, 78–84.

Bermudez, E. A., Rifai, N., Buring, J. E., Manson, J. E., & Ridker, P. M. (2002). Relation between markers of systemic vascular inflammation and smoking in women. *American Journal of Cardiology*, *89*, 1117–1119.

Blann, A. D., Steele, C., & McCollum, C. N. (1997). The influence of smoking and of oral and transdermal nicotine on blood pressure, and haematology and coagulation indices. *Thrombosis and Haemostasis*, *78*, 1093–1096.

Bofetta, P., Tredaniel, J., & Greco, A. (2000). Risk of childhood cancer and adult lung cancer after childhood exposure to passive smoke: A meta-analysis. *Environmental Health Perspectives*, *108*, 73–82.

Boysen, G., & Hecht, S. S. (2003). Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. *Mutation Research*, *543*, 17–30.

Bruner, J. P., & Forouzan, I. (1991). Smoking and buccally administered nicotine. Acute effect on uterine and umbilical artery Doppler flow velocity waveforms. *Journal of Reproductive Medicine*, *36*, 435–440.

Bucchioni, E., Kharitonov, S. A., Allegra, L., & Barnes, P. J. (2003). High levels of interleukin-6 in the exhaled breath condensate of patients with COPD. *Respiratory Medicine*, *97*, 1299–1302.

Burke, A., & Fitzgerald, G. A. (2003). Oxidative stress and smoking-induced vascular injury. *Progress in Cardiovascular Disease*, *46*, 79–90.

Burns, D. (2003). Epidemiology of smoking-induced cardiovascular disease. *Progress in Cardiovascular Disease*, *46*, 11–29.

Byrd, G. D., & Ogden, M. W. (2003). Liquid chromatographic/tandem mass spectrometric method for the determination of the tobacco-specific nitrosamine metabolite NNAL in smokers' urine. *Journal of Mass Spectrometry*, *38*, 98–107.

Carmella, S. G., Akerkar, S., & Hecht, S. S. (1993). Metabolites of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers' urine. *Cancer Research*, *53*, 721–724.

Carmella, S. G., Chen, M., Villalta, P. W., Gurney, J. G., Hatsukami, D., & Hecht, S. S. (2002). Ethylation and methylation of hemoglobin in smokers and non-smokers. *Carcinogenesis*, *23*, 1903–1910.

Carmella, S. G., Han, S., Fristad, A., Yang, Y., & Hecht, S. (2003). Analysis of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) in human urine. *Cancer Epidemiology, Biomarkers, & Prevention*, *12*, 1257–1261.

Carpagnano, G. E., Kharitonov, S. A., Foschino-Barbaro, M. P., Resta, O., Gramiccioni, E., & Barnes, P. J. (2003). Increased inflammatory markers in the exhaled breath condensate of cigarette smokers. *European Respiratory Journal*, *21*, 589–593.

Castelao, J. E., Yuan, J. M., Skipper, P. L., Tannenbaum, S. R., Gago-Dominguez, M., Crowder, J. S., Ross, R. K., & Yu, M. C. (2001). Gender- and smoking-related bladder cancer risk. *Journal of the National Cancer Institute*, *93*, 538–545.

Castro, L. C., Allen, R., Ogunyemi, D., Roll, K., & Platt, L. D. (1993). Cigarette smoking during pregnancy: Acute effects on uterine flow velocity waveforms. *Obstetrics and Gynecology*, *81*, 551–555.

Chalmers, G. W., MacLeod, K. J., Thomson, L., Little, S. A., McSharry, C., & Thomson, N. C. (2001). Smoking and airway inflammation in patients with mild asthma. *Chest*, *120*, 1917–1922.

Christensen, A. E., Tobiassen, M., Jensen, T. K., Wielandt, H., Bakketeig, L. S., & Host, A. (2004). Repeated validation of parental self-report smoking during pregnancy and infancy: A prospective cohort study of infants at high risk for allergy development. *Paediatric and Perinatal Epidemiology*, *18*, 73–79.

Cliver, S. P., Goldenberg, R. L., Cutter, G. R., Hoffman, H. J., Davis, R. O., & Nelson, K. G. (1995). The effect of cigarette smoking on neonatal anthropometric measurements. *Obstetrics and Gynecology*, *85*, 625–630.

Coghlin, J., Gann, P. H., Hammond, S. K., Skipper, P. L., Taghizadeh, K., Paul, M., & Tannenbaum, S. R. (1991). 4-Aminobiphenyl hemoglobin adducts in fetuses exposed to the tobacco smoke carcinogen in utero. *Journal of the National Cancer Institute*, *83*, 274–280.

Cogswell, M. E., Weisberg, P., & Spong, C. (2003). Cigarette smoking, alcohol use and adverse pregnancy outcomes: Implications for micronutrient supplementation. *Journal of Nutrition*, *133*(5 Suppl 2), 1722S–1731S.

Cooke, J. P. (2000). Does ADMA cause endothelial dysfunction? *Arteriosclerosis, Thrombosis, and Vascular Biology*, *20*, 2032–2037.

Corradi, M., Pesci, A., Casana, R., Alinovi, R., Goldoni, M., Vettori, M. V., & Cuomo, A. (2003). Nitrate in exhaled breath condensate of patients with different airway diseases. *Nitric Oxide*, *8*, 26–30.

Corradi, M., Rubinstein, I., Andreoli, R., Manini, P., Caglieri, A., Poli, D., Alinovi, R., & Mutti, A. (2003). Aldehydes in exhaled breath condensate of patients with chronic obstructive pulmonary disease. *American Journal of Respiratory and Critical Care Medicine*, *167*, 1301–1302.

Czernin, J., & Waldherr, C. (2003). Cigarette smoking and coronary blood flow. *Progress in Cardiovascular Disease*, *45*, 395–404.

Dadak, C., Kefalides, A., Sinzinger, H., & Weber, G. (1982). Reduced umbilical artery prostacyclin formation in complicated pregnancies. *American Journal of Obstetrics and Gynecology*, *144*, 792–795.

de Groot, E., Hovingh, G. K., Wiegman, A., Duriez, P., Smit, A. J., Fruchart, J. C., & Kastelein, J. J. (2004). Measurement of arterial wall thickness as a surrogate marker for atherosclerosis. *Circulation*, *109*(23 Suppl. 1), i1133–i1138.

Dempsey, D. A., & Benowitz, N. L. (2001). Risks and benefits of nicotine to aid smoking cessation in pregnancy. *Drug Safety*, *24*, 277–322.

Derauf, C., Katz, A. R., & Easa, D. (2003). Agreement between maternal self-reported ethanol intake and tobacco use during pregnancy and meconium assays for fatty acid ethyl esters and cotinine. *American Journal of Epidemiology*, *158*, 705–709.

Dezateux, C., Stocks, J., Wade, A. M., Dundas, I., & Fletcher, M. E. (2001). Airway function at one year: Association with premorbid airway function, wheezing, and maternal smoking. *Thorax*, *56*, 680–686.

DiFranza, J. R., Aligne, C. A., & Weitzman, M. (2004). Prenatal and postnatal environmental tobacco smoke exposure and children's health. *Pediatrics*, *113*(4 Suppl.), 1007–1015.

Dippolito, R., Foresi, A., Chetta, A., Castagnaro, A., Malorgio, R., Marangio, E., & Olivieri, D. (2001). Eosinophils in induced sputum from asymptomatic smokers with normal lung function. *Respiratory Medicine*, *95*, 969–974.

Effros, R. M., Hoagland, K. W., Bosbous, M., Castillo, D., Foss, B., Dunning, M., Gare, M., Lin, W., & Sun, F. (2002). Dilution of respiratory solutes in exhaled condensates. *American Journal of Respiratory and Critical Care Medicine*, *165*, 663–669.

Ehrlich, R. I., Du Toit, D., Jordaan, E., Zwarenstein, M., Potter, P., Volmink, J. A., & Weinberg, E. (1996). Risk factors for childhood asthma and wheezing. Importance of maternal and household smoking. *American Journal of Respiratory and Critical Care Medicine*, *154*(3 Pt. 1), 681–688.

Eliasson, B. (2003). Cigarette smoking and diabetes. *Progress in Cardiovascular Disease*, *45*, 405–413.

Eliasson, B., Attvall, S., Taskinen, M. R., & Smith, U. (1997). Smoking cessation improves insulin sensitivity in healthy middle-aged men. *European Journal of Clinical Investigation*, *27*, 450–456.

Eliopoulos, C., Klein, J., Phan, M. K., Knie, B., Greenwald, M., Chitayat, D., & Koren, G. (1994). Hair concentrations of nicotine and cotinine in women and their newborn infants. *The Journal of the American Medical Association*, *271*, 621–623.

Elliot, J. G., Carroll, N. G., James, A. L., & Robinson, P. J. (2003). Airway alveolar attachment points and exposure to cigarette smoke in utero. *American Journal of Respiratory and Critical Care Medicine*, *167*, 45–49.

Fahy, J. V., Liu, J., Wong, H., & Boushey, H. A. (1993). Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. *The American Review of Respiratory Disease*, *147*, 1126–1131.

Falcon, M., Vinas, P., Osuna, E., & Luna, A. (2002). Environmental exposures to lead and cadmium measured in human placenta. *Archives of Environmental Health*, *57*, 598–602.

Falter, B., Kutzer, C., & Richter, E. (1994). Biomonitoring of hemoglobin adducts: Aromatic amines and tobacco-specific nitrosamines. *The Clinical Investigator*, *72*, 364–371.

Fennell, T. R., MacNeela, J. P., Morris, R. W., Watson, M., Thompson, C. L., & Bell, D. A. (2000). Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: Effects of glutathione S-transferase T1-null and M1-null genotypes. *Cancer Epidemiology, Biomarkers, & Prevention*, *9*, 705–712.

Fried, P. A., & Makin, J. E. (1987). Neonatal behavioural correlates of prenatal exposure to marihuana, cigarettes and alcohol in a low risk population. *Neurotoxicology and Teratology*, *9*, 1–7.

Fried, P. A., & Watkinson, B. (1988). 12- and 24-month neurobehavioural follow-up of children prenatally exposed to marihuana, cigarettes and alcohol. *Neurotoxicology and Teratology*, *10*, 305–313.

Fried, P. A., & Watkinson, B. (2001). Differential effects on facets of attention in adolescents prenatally exposed to cigarettes and marihuana. *Neurotoxicology and Teratology*, *23*, 421–430.

Fried, P. A., Watkinson, B., Dillon, R. F., & Dulberg, C. S. (1987). Neonatal neurological status in a low-risk population after prenatal exposure to cigarettes, marijuana, and alcohol. *Journal of Developmental and Behavioral Pediatrics*, *8*, 318–326.

Fried, P. A., Watkinson, B., & Gray, R. (1998). Differential effects on cognitive functioning in 9- to 12-year olds prenatally exposed to cigarettes and marihuana. *Neurotoxicology and Teratology*, *20*, 293–306.

Fried, P. A., Watkinson, B., & Siegel, L. S. (1997). Reading and language in 9- to 12-year olds prenatally exposed to cigarettes and marijuana. *Neurotoxicology and Teratology*, *19*, 171–183.

Garey, K. W., Neuhauser, M. M., Robbins, R. A., Danziger, L. H., & Rubinstein, I. (2004). Markers of inflammation in exhaled breath condensate of young healthy smokers. *Chest*, *125*, 22–26.

Gilliland, F. D., Berhane, K., Li, Y. F., Rappaport, E. B., & Peters, J. M. (2003). Effects of early onset asthma and in utero exposure to maternal smoking on childhood lung function. *American Journal of Respiratory and Critical Care Medicine*, *167*, 917–924.

Gilliland, F. D., Berhane, K., McConnell, R., Gauderman, W. J., Vora, H., Rappaport, E. B., Avol, E., & Peters, J. M. (2000). Maternal smoking during pregnancy, environmental tobacco smoke exposure and childhood lung function. *Thorax*, *55*, 271–276.

Goldenberg, R. L., Davis, R. O., Cliver, S. P., Cutter, G. R., Hoffman, H. J., Dubard, M. B., & Copper, R. L. (1993). Maternal risk factors and their influence on fetal anthropometric measurements. *American Journal of Obstetrics and Gynecology*, *168*, 1197–1203; discussion, 1203–1195.

Hamrick, S. E., & Ferriero, D. M. (2003). The injury response in the term newborn brain: Can we neuroprotect? *Current Opinion in Neurology*, *16*, 147–154.

Hargreave, F. E. (1999). Induced sputum for the investigation of airway inflammation: Evidence for its clinical application. *Canadian Respiratory Journal*, *6*, 169–174.

Harrison, K. L., & Robinson, A. G. (1981). The effect of maternal smoking on carboxyhemoglobin levels and acid-base balance of the fetus. *Clinical Toxicology*, *18*, 165–168.

Hatsukami, D., Giovino, G., Eissenberg, T., Clark, P., Lawrence, D., & Leischow, S. (2005). Methods to assess potential reduced exposure products. *Nicotine & Tobacco Research*, *7*, 827–844.

Hatsukami, D., Hecht, S., Hennrikus, D., Joseph, A., & Pentel, P. (2003). Biomarkers of tobacco exposure or harm: Application to clinical and epidemiological studies. *Nicotine & Tobacco Research*, *5*, 387–396.

Hatsukami, D., Lemmonds, C. A., Zhang, Y., Murphy, S. E., Le, C., Carmella, S. G., & Hecht, S. S. (2004). Evaluation of carcinogen exposure in people who used "reduced exposure" tobacco products. *Journal of the National Cancer Institute*, *96*, 844–852.

Hatsukami, D., Slade, J., Benowitz, N., Giovino, G., Gritz, E., Leischow, S., & Warner, K. (2002). Reducing tobacco harm: Research challenges and issues. *Nicotine & Tobacco Research*, *4*(Suppl. 2), S89–S101.

Hattotuwa, K., Gamble, E. A., O'Shaughnessy, T., Jeffery, P. K., & Barnes, N. C. (2002). Safety of bronchoscopy, biopsy, and BAL in research patients with COPD. *Chest*, *122*, 1909–1912.

Hecht, S. S. (1998). Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. *Chemical Research in Toxicology*, *11*, 559–603.

Hecht, S. S. (2002). Human urinary carcinogen metabolites: Biomarkers for investigating tobacco and cancer. *Carcinogenesis*, *23*, 907–922.

Hecht, S. S. (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nature Reviews. *Cancer*, *3*, 733–744.

Hecht, S. S., Carmella, S. G., Chen, M., Koch, J. D., Miller, A., Murphy, S. E., Jensen, J. A., Zimmerman, C. L., & Hatsukami, D. K. (1999). Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. *Cancer Research*, *59*, 590–596.

Hecht, S. S., Carmella, S. G., Le, K., Murphy, S. E., Li, Y. S., Le, C., Jensen, J., & Hatsukami, D. (2004). Effects of reduced cigarette smoking on levels of 1-hydroxypyrene in urine. *Cancer Epidemiology, Biomarkers, & Prevention*, *13*, 834–842.

Hecht, S. S., Carmella, S. G., & Murphy, S. E. (1994). Tobacco-specific nitrosamine-hemoglobin adducts. *Methods in Enzymology*, *231*, 657–667.

Hecht, S. S., Murphy, S. E., Carmella, S. G., Zimmerman, C. L., Losey, L., Kramarczuk, I., Roe, M. R., Puumala, S. S., Li, Y. S., Le, C. T., Jensen, J., & Hatsukami, D. K. (2004). Effects of reduced cigarette smoking on uptake of a tobacco-specific lung carcinogen. *Journal of the National Cancer Institute*, *96*, 107–115.

Hill, A. T., Bayley, D. L., Campbell, E. J., Hill, S. L., & Stockley, R. A. (2000). Airways inflammation in chronic bronchitis: The effects of smoking and alpha1-antitrypsin deficiency. *European Respiratory Journal*, *15*, 886–890.

Hogman, M., Holmkvist, T., Walinder, R., Merilainen, P., Ludviksdottir, D., Hakansson, L., & Hedenstrom, H. (2002). Increased nitric oxide elimination from the airways after smoking cessation. *Clinical Science*, *103*, 15–19.

International Agency for Research on Cancer. (2004). Tobacco smoke and involuntary smoking. In, *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. (Vol. 83). Lyon, France: Author.

Isawa, T., Teshima, T., Hirano, T., Ebina, A., & Konno, K. (1984). Mucociliary clearance mechanism in smoking and nonsmoking normal subjects. *Journal of Nuclear Medicine*, *25*, 352–359.

Jacob III, P., Hatsukami, D., Severson, H., Hall, S., Yu, L., & Benowitz, N. (2002). Anabasine and anatabine as biomarkers for tobacco use during nicotine replacement therapy. *Cancer Epidemiology, Biomarkers, & Prevention*, *11*, 1668–1673.

Jeffery, P., Holgate, S., & Wenzel, S. (2003). Methods for the assessment of endobronchial biopsies in clinical research:

Application to studies of pathogenesis and the effects of treatment. *American Journal of Respiratory and Critical Care Medicine*, *168*(6 Pt. 2), S1–S17.

Jensen, E. J., Pedersen, B., Frederiksen, R., & Dahl, R. (1998). Prospective study on the effect of smoking and nicotine substitution on leucocyte blood counts and relation between blood leucocytes and lung function. *Thorax*, *53*, 784–789.

Jongeneelen, F. J. (2001). Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to polycyclic aromatic hydrocarbons. *The Annals of Occupational Hygiene*, *45*, 3–13.

Joseph, A. M., Hecht, S. S., Murphy, S. E., Carmella, S. G., Le, C. T., Zhang, Y., Han, S., & Hatsukami, D. K. (2005). Relationships between cigarette consumption and biomarkers of tobacco toxin exposure. *Cancer Epidemiology, Biomarkers, and Prevention*, *14*, 2963–2968.

Kaijser, M., Granath, F., Jacobsen, G., Cnattingius, S., & Ekbom, A. (2000). Maternal pregnancy estriol levels in relation to anamnestic and fetal anthropometric data. *Epidemiology*, *11*, 315–319.

Kandel, D. B., Wu, P., & Davies, M. (1994). Maternal smoking during pregnancy and smoking by adolescent daughters. *American Journal of Public Health*, *84*, 1407–1413.

Kavuru, M. S., Dweik, R. A., & Thomassen, M. J. (1999). Role of bronchoscopy in asthma research. *Clinics in Chest Medicine*, *20*, 153–189.

Keatings, V. M., Collins, P. D., Scott, D. M., & Barnes, P. J. (1996). Differences in interleukin-8 and tumor necrosis factor-alphia in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *American Journal of Respiratory and Critical Care Medicine*, *153*, 530–534.

Kennedy, S. M., Elwood, R. K., Wiggs, B. J., Pare, P. D., & Hogg, J. C. (1984). Increased airway mucosal permeability of smokers. Relationship to airway reactivity. *The American Review of Respiratory Disease*, *129*, 143–148.

Kharitonov, S. A., & Barnes, P. J. (2001). Exhaled markers of inflammation. *Current Opinion in Allergy and Clinical Immunology*, *1*, 217–224.

Kharitonov, S. A., & Barnes, P. J. (2002). Biomarkers of some pulmonary diseases in exhaled breath. *Biomarkers*, *7*, 1–32.

Khuri, F. R., Lee, J. S., Lippman, S. M., Lee, J. J., Kalapurakal, S., Yu, R., Ro, J. Y., Morice, R. C., Hong, W. K., & Hittelman, W. N. (2001). Modulation of proliferating cell nuclear antigen in the bronchial epithelium of smokers. *Cancer Epidemiology, Biomarkers, & Prevention*, *10*, 311–318.

Klech, H., & Pohl, W. (1989). Technical recommendations and guidelines for bronchoalveolar lavage (BAL). Report of the S.E.P. Task Group on BAL. *European Respiratory Journal*, *2*, 561–585.

Kondo, T., Hayashi, M., Takeshita, K., Numaguchi, Y., Kobayashi, K., Iino, S., Inden, Y., & Murohara, T. (2004). Smoking cessation rapidly increases circulating progenitor cells in peripheral blood in chronic smokers. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*, 1–6.

Kooi, E. M., Vrijlandt, E. J., Boezen, H. M., & Duiverman, E. J. (2004). Children with smoking parents have a higher airway resistance measured by the interruption technique. *Pediatric Pulmonology*, *38*, 419–424.

Kriek, E., Rojas, M., Alexandrov, K., & Bartsch, H. (1998). Polycyclic aromatic hydrocarbon-DNA adducts in humans: Relevance as biomarkers for exposure and cancer risk. *Mutation Research*, *400*, 215–231.

Lackmann, G. M., Salzberger, U., Tollner, U., Chen, M., Carmella, S. G., & Hecht, S. S. (1999). Metabolites of a tobacco-specific carcinogen in urine from newborns. *Journal of the National Cancer Institute*, *91*, 459–465.

Lain, K. Y., Wilson, J. W., Crombleholme, W. R., Ness, R. B., & Roberts, J. M. (2003). Smoking during pregnancy is associated with alterations in markers of endothelial function. *American Journal of Obstetrics and Gynecology*, *189*, 1196–1201.

Lam, S., leRiche, J. C., Zheng, Y., Coldman, A., MacAulay, C., Hawk, E., Kelloff, G., & Gazdar, A. F. (1999). Sex-related differences in bronchial epithelial changes associated with tobacco smoking. *Journal of the National Cancer Institute*, *91*, 691–696.

Lam, S., MacAulay, C., Le Riche J., C., Dyachkova, Y., Coldman, A., Guillaud, M., Hawk, E., Christen, M. O., & Gazdar, A. F. (2002). A randomized phase IIb trial of anethole dithiolethione in smokers with bronchial dysplasia. *Journal of the National Cancer Institute*, *94*, 1001–1009.

Larsen, L. G., Clausen, H. V., & Jonsson, L. (2002). Stereologic examination of placentas from mothers who smoke during pregnancy. *American Journal of Obstetrics and Gynecology*, *186*, 531–537.

Lee, J. S., Lippman, S. M., Benner, S. E., Lee, J. J., Ro, J. Y., Lukeman, J. M., Morice, R. C., Peters, E. J., Pang, A. C., & Fritsche, H. A., Jr. (1994). Randomized placebo-controlled trial of isotretinoin in chemoprevention of bronchial squamous metaplasia. *Journal of Clinical Oncology*, *12*, 937–945.

Ley, K. (2003). The role of selectins in inflammation and disease. *Trends in Molecular Medicine*, *9*, 263–268.

Li, C. Q., Windsor, R. A., Perkins, L., Goldenberg, R. L., & Lowe, J. B. (1993). The impact on infant birth weight and gestational age of cotinine-validated smoking reduction during pregnancy. *The Journal of the American Medical Association*, *269*, 1519–1524.

Lin, L. C., Tyan, Y. C., Shih, T. S., Chang, Y. C., & Liao, P. C. (2004). Development and validation of an isotope-dilution electrospray ionization tandem mass spectrometry method with an on-line sample clean-up device for the quantitative analysis of the benzene exposure biomarker S-phenylmercapturic acid in human urine. *Rapid Communications in Mass Spectrometry*, *18*, 1310–1316.

Lindblad, A., Marsal, K., & Andersson, K. E. (1988). Effect of nicotine on human fetal blood flow. *Obstetrics and Gynecology*, *72*(3 Pt. 1), 371–382.

Linder, J., & Rennard, S. I. (1988). *Atlas of bronchoalveolar lavage*. Chicago: American Society of Clinical Pathology Press.

Linnet, K. M., Dalsgaard, S., Obel, C., Wisborg, K., Henriksen, T. B., Rodriguez, A., Kotimaa, A., Moilanen, I., Thomsen, P. H., Olsen, J., & Jarvelin, M. R. (2003). Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: Review of the current evidence. *American Journal of Psychiatry*, *160*, 1028–1040.

Lykkesfeldt, J., Christen, S., Wallock, L. M., Chang, H. H., Jacob, R. A., & Ames, B. N. (2000). Ascorbate is depleted by smoking and repleted by moderate supplementation: A study in male smokers and nonsmokers with matched dietary antioxidant intakes. *American Journal of Clinical Nutrition*, *71*, 530–536.

Lynch, A. M., & Bruce, N. W. (1989). Placental growth in rats exposed to carbon monoxide at selected stages of pregnancy. *Biology of the Neonate*, *56*, 151–157.

MacArthur, C., & Knox, E. G. (1988). Smoking in pregnancy: Effects of stopping at different stages. *British Journal of Obstetrics & Gynaecology*, *95*, 551–555.

Maclure, M., Bryant, M. S., Skipper, P. L., & Tannenbaum, S. R. (1990). Decline of the hemoglobin adduct of 4-aminobiphenyl during withdrawal from smoking. *Cancer Research*, *50*, 181–184.

Mactutus, C. F. (1989). Developmental neurotoxicity of nicotine, carbon monoxide, and other tobacco smoke constituents. *Annals of the New York Academy of Sciences*, *562*, 105–122.

Maestri, L., Negri, S., Ferrari, M., Ghittori, S., & Imbriani, M. (2005). Determination of urinary S-phenylmercapturic acid, a specific metabolite of benzene, by liquid chromatography/single quadrupole mass spectrometry. *Rapid Communications in Mass Spectrometry*, *19*, 1139–1144.

Mahmarian, J., Moye, L., Nasser, G., Nagueh, S., Bloom, M., Benowitz, N. L., Verani, M. S., Byrd, W. G., & Pratt, C. M. (1997). Nicotine patch therapy in smoking cessation reduces the extent of exercise-induced myocardial ischemia. *Journal of the American College of Cardiology*, *30*, 125–130.

Marques, L. J., Teschler, H., Guzman, J., & Costabel, U. (1997). Smoker's lung transplanted to a nonsmoker. Long-term detection of smoker's macrophages. *American Journal of Respiratory and Critical Care Medicine*, *156*, 1700–1702.

McCartney, J. S., Fried, P. A., & Watkinson, B. (1994). Central auditory processing in school-age children prenatally exposed to cigarette smoke. *Neurotoxicology and Teratology*, *16*, 269–276.

McMahon, M. J., Brown, H. L., & Dean, R. A. (1997). Umbilical cord thiocyanate and thyroid function in intrauterine growth-restricted infants of the smoking gravida. *Journal of Perinatology*, *17*, 370–374.

Melikian, A. A., Qu, Q., Shore, R., Li, G., Li, H., Jin, X., Cohen, B., Chen, L., Li, Y., Yin, S., Mu, R., Zhang, X., & Wang, Y. (2002). Personal exposure to different levels of benzene and its relationships to the urinary metabolites S-phenylmercapturic acid and trans,-trans-muconic acid. *Journal of Chromatography. B. Analytical Technologies in the Biomedical and Life Sciences*, *778*, 211–221.

Metherate, R., & Hsieh, C. Y. (2003). Regulation of glutamate synapses by nicotinic acetylcholine receptors in auditory cortex. *Neurobiology of Learning and Memory*, *80*, 285–290.

Milerad, J., Vege, A., Opdal, S. H., & Rognum, T. O. (1998). Objective measurements of nicotine exposure in victims of sudden infant death syndrome and in other unexpected child deaths. *Journal of Pediatrics*, *133*, 232–236.

Milunsky, A., Carmella, S. G., Ye, M., & Hecht, S. S. (2000). A tobacco-specific carcinogen in the fetus. *Prenatal Diagnosis*, *20*, 307–310.

Minty, B. D., Jordan, C., & Jones, J. G. (1981). Rapid improvement in abnormal pulmonary epithelial permeability after stopping cigarettes. *British Medical Journal*, *282*, 1183–1186.

Mio, T., Romberger, D. J., Robbins, R. A., Heires, A., & Rennard, S. I. (1997). Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. *American Journal of Respiratory and Critical Care Medicine*, *155*, 1770–1776.

Moller, P., Knudsen, L. E., Loft, S., & Wallin, H. (2000). The comet assay as a rapid test in biomonitoring occupational exposure to DNA-damaging agents and effect of confounding factors. *Cancer Epidemiology, Biomarkers, & Prevention*, *9*, 1005–1015.

Montuschi, P., Collins, J. V., Ciabattoni, G., Lazzeri, N., Corradi, M., Kharitonov, S. A., & Barnes, P. J. (2000). Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. *American Journal of Respiratory and Critical Care Medicine*, *162*(3 Pt. 1), 1175–1177.

Morrow, J. D., Frei, B., Longmire, A. W., Gaziano, J. M., Lynch, S. M., Shyr, Y., Strauss, W. E., Oates, J. A., & Roberts, L. J., 2nd. (1995). Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *The New England Journal of Medicine*, *332*, 1198–1203.

Morrow, R. J., Ritchie, J. W., & Bull, S. B. (1988). Maternal cigarette smoking: The effects on umbilical and uterine blood flow velocity. *American Journal of Obstetrics and Gynecology*, *159*, 1069–1071.

Myers, S. R., Spinnato, J. A., Pinorini-Godly, M. T., Cook, C., Boles, B., & Rodgers, G. C. (1996). Characterization of 4-aminobiphenyl-hemoglobin adducts in maternal and fetal blood-samples. *Journal of Toxicology and Environmental Health*, *47*, 553–566.

Naeye, R. L. (1978). Effects of maternal cigarette smoking on the fetus and placenta. *British Journal of Obstetrics & Gynaecology*, *85*, 732–737.

Naeye, R. L., & Peters, E. C. (1984). Mental development of children whose mothers smoked during pregnancy. *Obstetrics and Gynecology*, *64*, 601–607.

NHLB/WHO Workshop Panel. (2003). Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. In *Global initiative for chronic obstructive lung disease. Executive summary*. Retrieved 2003 from www.goldcopd.com

Niewoehner, D. E., Kleinerman, J., & Rice, D. B. (1974). Pathologic changes in the peripheral airways of young cigarette smokers. *The New England Journal of Medicine*, *291*, 755–758.

Nowak, D., Kalucka, S., Bialasiewicz, P., & Krol, M. (2001). Exhalation of H2O2 and thiobarbituric acid reactive substances (TBARs) by healthy subjects. *Free Radical Biology & Medicine*, *30*, 178–186.

Nowak, J., Murray, J. J., Oates, J. A., & FitzGerald, G. A. (1987). Biochemical evidence of a chronic abnormality in platelet and vascular function in healthy individuals who smoke cigarettes. *Circulation*, *76*, 6–14.

Obwegeser, R., Oguogho, A., Ulm, M., Berghammer, P., & Sinzinger, H. (1999). Maternal cigarette smoking increases F2-isoprostanes and reduces prostacyclin and nitric oxide in umbilical vessels. *Prostaglandins and Other Lipid Mediators*, *57*, 269–279.

Ogburn, P. L., Jr., Hurt, R. D., Croghan, I. T., Schroeder, D. R., Ramin, K. D., Offord, K. P., & Moyer, T. P. (1999). Nicotine patch use in pregnant smokers: Nicotine and cotinine levels and fetal effects. *American Journal of Obstetrics and Gynecology*, *181*, 736–743.

Ohwada, A., Takahashi, H., Nagaoka, I., Iwabuchi, K., Mikami, O., & Kira, S. (1995). Effect of cigarette smoke on the mRNA and protein expression of carcinoembryonic antigen (CEA), a possible chemoattractant for neutrophils in human bronchioloalveolar tissues. *Thorax*, *50*, 651–657.

Olanow, C. W., & Arendash, G. W. (1984). Metals and free radicals in neurodegeneration. *Current Opinion in Neurology*, *7*, 548–558.

Oncken, C., Hardardottir, H., & Smeltzer, J. (1998). Human studies of nicotine replacement during pregnancy. In: N. Benowitz (Ed.), *Nicotine safety and toxicity* (pp. 107–116). New York: Oxford University Press.

Oncken, C., Hatsukami, D. K., Lupo, V. R., Lando, H. A., Gibeau, L. M., & Hansen, R. J. (1996). Effects of short-term use of nicotine gum in pregnant smokers. *Clinical Pharmacology and Therapeutics*, *59*, 654–661.

Oncken, C., Henry, K. M., Campbell, W. A., Kuhn, C. M., Slotkin, T. A., & Kranzler, H. R. (2003). Effect of maternal smoking on fetal catecholamine concentrations at birth. *Pediatric Research*, *53*, 119–124.

Ong, K. K., Preece, M. A., Emmett, P. M., Ahmed, M. L., & Dunger, D. B. (2002). Size at birth and early childhood growth in relation to maternal smoking, parity and infant breast-feeding: Longitudinal birth cohort study and analysis. *Pediatric Research*, *52*, 863–867.

Ostrea, E. M., Jr., Knapp, D. K., Romero, A., Montes, M., & Ostrea, A. R. (1994). Meconium analysis to assess fetal exposure to nicotine by active and passive maternal smoking. *Journal of Pediatrics*, *124*, 471–476.

Ozerol, E., Ozerol, I., Gokdeniz, R., Temel, I., & Akyol, O. (2004). Effect of smoking on serum concentrations of total homocysteine, folate, vitamin B12, and nitric oxide in pregnancy: A preliminary study. *Fetal Diagnosis and Therapy*, *19*, 145–148.

Panagiotakos, D. B., Pitsavos, C., Chrysohoou, C., Skoumas, J., Masoura, C., Toutouzas, P., & Stefanadis, C. (2004). Effect of exposure to secondhand smoke on markers of inflammation: The ATTICA study. *American Journal of Medicine*, *116*, 145–150.

Pavia, D., Thomson, M. L., & Pocock, S. J. (1971). Evidence for temporary slowing of mucociliary clearance in the lung caused by tobacco smoking. *Nature*, *231*, 325–326.

Pearson, T. A., Mensah, G. A., Alexander, R. W., Anderson, J. L., Cannon, R. O., 3rd., Criqui, M., Fadl, Y. Y., Fortmann, S. P., Hong, Y., Myers, G. L., Rifai, N., Smith, S. C., Jr., Taubert, K., Tracy, R. P., & Vinicor, F. (2003). Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation*, *107*, 499–511.

Perera, F. P., Jedrychowski, W., Rauh, V., & Whyatt, R. M. (1999). Molecular epidemiologic research on the effects of environmental pollutants on the fetus. *Environmental Health Perspectives*, *107*(Suppl. 3), 451–460.

Petays, T., von Hertzen, L., Metso, T., Rytila, P., Jousilahti, P., & Helenius, I. (2003). Smoking and atopy as determinants of sputum eosinophilia and bronchial hyper-responsiveness in adults with normal lung function. *Respiratory Medicine*, *97*, 947–954.

Petridou, E., Panagiotopoulou, K., Katsouyanni, K., Spanos, E., & Trichopoulos, D. (1990). Tobacco smoking, pregnancy estrogens, and birth weight. *Epidemiology*, *1*, 247–250.

Philipp, K., Pateisky, N., & Endler, M. (1984). Effects of smoking on uteroplacental blood flow. *Gynecologic and Obstetric Investigation*, *17*, 179–182.

Phillips, D. H. (2002). Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis*, *23*, 1979–2004.

Piasek, M., Blanusa, M., Kostial, K., & Laskey, J. W. (2001). Placental cadmium and progesterone concentrations in cigarette smokers. *Reproductive Toxicology*, *15*, 673–681.

Picone, T. A., Allen, L. H., Olsen, P. N., & Ferris, M. E. (1982). Pregnancy outcome in North American women. II. Effects of diet, cigarette smoking, stress, and weight gain on placentas, and on neonatal physical and behavioral characteristics. *American Journal of Clinical Nutrition*, *36*, 1214–1224.

Pignatelli, B., Li, C. Q., Boffetta, P., Chen, Q., Ahrens, W., Nyberg, F., Mukeria, A., Bruske-Hohlfeld, I., Fortes, C., Constantinescu, V., Ischiropoulos, H., & Ohshima, H. (2001). Nitrated and oxidized plasma proteins in smokers and lung cancer patients. *Cancer Research*, *61*, 778–784.

Pilz, H., Oguogho, A., Chehne, F., Lupattelli, G., Palumbo, B., & Sinzinger, H. (2000). Quitting cigarette smoking results in a fast improvement of in vivo oxidation injury (determined via plasma, serum and urinary isoprostane). *Thrombosis Research*, *99*, 209–221.

Prindiville, S. A., Byers, T., Hirsch, F. R., Franklin, W. A., Miller, Y. E., Vu, K. O., Wolf, H. J., Baron, A. E., Shroyer, K. R., Zeng, C., Kennedy, T. C., & Bunn, P. A. (2003). Sputum cytological atypia as a predictor of incident lung cancer in a cohort of heavy smokers

with airflow obstruction. *Cancer Epidemiology, Biomarkers, & Prevention*, *12*, 987–993.

Puranik, R., & Celermajer, D. S. (2003). Smoking and endothelial function. *Progress in Cardiovascular Disease*, *45*, 443–458.

Rennard, S. I., Daughton, D., Fujita, J., Oehlerking, M. B., Dobson, J. R., Stahl, M. G., Dobson, J. R., Stahl, M. G., Robbins, R. A., & Thompson, A. B. (1990). Short-term smoking reduction is associated with reduction in measures of lower respiratory tract inflammation in heavy smokers. *European Respiratory Journal*, *3*, 752–759.

Rennard, S. I., Daughton, D. M., Buchalter, S. E., Floreani, A. A., Larson, L., Millatmal, T., Robbins, R. A., Romberger, D. J., Sisson, J. H., Spurzem, J. R., Thompson, A. B., & Von Essen S., G. (1994). The influence of cigarette reduction or switching to lower nicotine yield cigarettes on measures of airway inflammation. *American Journal of Respiratory and Critical Care Medicine*, *149*, A395.

Rennard, S. I., Umino, T., Millatmal, T., Daughton, D. M., Manouilova, L. S., Ullrich, F. A., Patil, K. D., Romberger, D. J., Floreani, A. A., & Anderson, J. R. (2002). Evaluation of subclinical respiratory tract inflammation in heavy smokers who switch to a cigarette-like nicotine delivery device that primarily heats tobacco. *Nicotine & Tobacco Research*, *4*, 467–476.

Reynolds, H. Y. (1987). State of the art: Bronchoalveolar lavage. *The American Review of Respiratory Disease*, *135*, 250–263.

Robbins, R. A., Millatmal, T., Lassi, K., Rennard, S., & Daughton, D. (1997). Smoking cessation is associated with an increase in exhaled nitric oxide. *Chest*, *112*, 313–318.

Roth, M. D., Arora, A., Barsky, S. H., Kleerup, E. C., Simmons, M., & Tashkin, D. P. (1998). Airway inflammation in young marijuana and tobacco smokers. *American Journal of Respiratory and Critical Care Medicine*, *157*, 928–937.

Saareks, V., Ylitalo, P., Alanko, J., Mucha, I., & Riutta, A. (2001). Effects of smoking cessation and nicotine substitution on systemic eicosanoid production in man. *Naunyn-Schmiedeberg's Archive of Pharmacology*, *363*, 556–561.

Saetta, M., Ghezzo, H., Kim, W. D., King, M., Angus, G. E., Wang, N. -S., & Cosio, M. G. (1985). Loss of alveolar attachments in smokers. *The American Review of Respiratory Disease*, *132*, 894–900.

Sargeant, L. A., Khaw, K. T., Bingham, S., Day, N. E., Luben, R. N., Oakes, S., Welch, A., & Wareham, N. J. (2001). Cigarette smoking and glycaemia: The EPIC-Norfolk Study. European Prospective Investigation into Cancer. *International Journal of Epidemiology*, *30*, 547–554.

Saxton, D. W. (1978). The behaviour of infants whose mothers smoke in pregnancy. *Early Human Development*, *2*, 363–369.

Scherer, G., Renner, T., & Meger, M. (1998). Analysis and evaluation of trans,trans-muconic acid as a biomarker for benzene exposure. *Journal of Chromatography. B, Biomedical Sciences and Applications*, *717*, 179–199.

Scott, D. A., Stapleton, J. A., Wilson, R. F., Sutherland, G., Palmer, R. M., Coward, P. Y., & Gustavsson, G. (2000). Dramatic decline in circulating intercellular adhesion molecule-1 concentration on quitting tobacco smoking. *Blood Cells, Molecules & Diseases*, *26*, 255–258.

Secker-Walker, R. H., Vacek, P. M., Flynn, B. S., & Mead, P. B. (1997a). Exhaled carbon monoxide and urinary cotinine as measures of smoking in pregnancy. *Addictive Behaviors*, *22*, 671–684.

Secker-Walker, R. H., Vacek, P. M., Flynn, B. S., & Mead, P. B. (1997b). Smoking in pregnancy, exhaled carbon monoxide, and birth weight. *Obstetrics and Gynecology*, *89*(5 Pt. 1), 648–653.

Sexton, M., Fox, N. L., & Hebel, J. R. (1990). Prenatal exposure to tobacco: II. Effects on cognitive functioning at age three. *International Journal of Epidemiology*, *19*, 72–77.

Sexton, M., & Hebel, J. R. (1984). A clinical trial of change in maternal smoking and its effect on birth weight. *The Journal of the American Medical Association*, *251*, 911–915.

Sherrill, D. L., Lebowitz, M. D., Knudson, R. J., & Burrows, B. (1991). Smoking and symptom effects on the curves of lung function growth and decline. *The American Review of Respiratory Disease*, *144*, 17–22.

Shields, P. (2002). Tobacco smoking, harm reduction and biomarkers. *Journal of the National Cancer Institute*, *94*, 1435–1444.

Simpson, A. J., Gray, R. S., Moore, N. R., & Booth, N. A. (1997). The effects of chronic smoking on the fibrinolytic potential of plasma and platelets. *British Journal of Haematology*, *97*, 208–213.

Skipper, P. L., & Tannenbaum, S. R. (1990). Protein adducts in the molecular dosimetry of chemical carcinogens. *Carcinogenesis*, *11*, 507–518.

Skold, C. M., Eklund, A., Hed, J., & Hernbrand, R. (1992). Endocytosis of cigarette-smoke condensate by rabbit alveolar macrophages in vitro measured as fluorescence intensity. *European Respiratory Journal*, *5*, 53–58.

Skold, C. M., Hed, J., & Eklund, A. (1992). Smoking cessation rapidly reduces cell recovery in bronchoalveolar lavage fluid, while alveolar macrophage fluorescence remains high. *Chest*, *101*, 989–995.

Slotkin, T. A., Lappi, S. E., McCook, E. C., Lorber, B. A., & Seidler, F. J. (1995). Loss of neonatal hypoxia tolerance after prenatal nicotine exposure: Implications for sudden infant death syndrome. *Brain Research Bulletin*, *38*, 69–75.

Spurzem, J. R., Thompson, A. B., Daughton, D. M., Mueller, M., Linder, J., & Rennard, S. I. (1991). Chronic inflammation is associated with an increased proportion of goblet cells recovered by bronchial lavage. *Chest*, *100*, 389–393.

SRNT Subcommittee on Biochemical Verification. (2002). Biochemical verification of tobacco use and cessation. *Nicotine & Tobacco Research*, *4*, 149–159.

Stick, S. M., Burton, P. R., Gurrin, L., Sly, P. D., & Le Sour P., N. (1996). Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. *Lancet*, *348*, 1060–1064.

Stockley, R. A., Shaw, J., Whitfield, A. G., Whitehead, T. P., Clarke, C. A., & Burnett, D. (1986). Effect of cigarette smoking, pulmonary inflammation, and lung disease on concentrations of carcinoembryonic antigen in serum and secretions. *Thorax*, *41*, 17–24.

Stratton, K., Shetty, P., Wallace, R., & Bondurant, S., (Eds.). (2001). *Clearing the smoke: Assessing the science base for tobacco harm reduction*. Institute of Medicine. Washington, DC: National Academies Press.

Stubbe, I., Eskilsson, J., & Nilsson-Ehle, P. (1982). High-density lipoprotein concentrations increase after stopping smoking. *British Medical Journal*, *284*, 1511–1513.

Svanes, C., Omenaas, E., Jarvis, D., Chinn, S., Gulsvik, A., & Burney, P. (2004). Parental smoking in childhood and adult obstructive lung disease: Results from the European Community Respiratory Health Survey. *Thorax*, *59*, 295–302.

Swan, G. E., Hodgkin, J. E., Roby, T., Mittman, C., Jacobo, N., & Peters, J. (1992). Reversibility of airways injury over a 12-month period following smoking cessation. *Chest*, *101*, 607–612.

Tager, I., Segal, M., Speizer, F., & Weiss, S. (1988). The natural history of forced expiratory volumes. *The American Review of Respiratory Disease*, *138*, 837–849.

Tashkin, D. P. (2002). The role of small airway inflammation in asthma. *Allergy and Asthma Proceedings*, *23*, 233–242.

Tharnpoophasiam, P., Kongtip, P., Wongwit, W., Fungladda, W., & Kitayaporn, D. (2004). Simultaneous determination of trans, transmuconic acid and s-phenylmercapturic acid by high pressure liquid chromatography and its application. *The Southeast Asian Journal of Tropical Medicine and Public Health*, *35*, 717–723.

Thompson, A. B., Daughton, D., Robbins, R. A., Ghafouri, M. A., Oehlerking, M., & Rennard, S. I. (1989). Intraluminal airway inflammation in chronic bronchitis. Characterization and correlation with clinical parameters. *The American Review of Respiratory Disease*, *140*, 1527–1537.

Thompson, A. B., Huerta, G., Robbins, R. A., Sisson, J. H., Spurzem, J. R., von Essen, S., Rickard, K. A., Romberger, D. J., Rubinstein, I., Ghafouri, M., Daughton, D., & Rennard, S. I. (1993). The bronchitis index. A semiquantitative visual scale = for the assessment of airways inflammation. *Chest*, *103*, 1482–1488.

Traves, S. L., Culpitt, S. V., Russell, R. E., Barnes, P. J., & Donnelly, L. E. (2002). Increased levels of the chemokines GROalpha and MCP-1 in sputum samples from patients with COPD. *Thorax*, *57*, 590–595.

U.S. Department of Health & Human Services. (2001). *Women and smoking: A report of the surgeon general. Reproductive outcomes*. Washington, DC: Author, Public Health Service, Office of the Surgeon General.

U.S. Department of Health & Human Services. (2004). *The health consequences of smoking: A report of the surgeon general*. Atlanta, GA: Author, Centers for Disease Control and Prevention, National

Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Ulm, M. R., Plockinger, B., Pirich, C., Gryglewski, R. J., & Sinzinger, H. F. (1995). Umbilical arteries of babies born to cigarette smokers generate less prostacyclin and contain less arginine and citrulline compared with those of babies born to control subjects. *American Journal of Obstetrics and Gynecology*, *172*, 1485–1487.

Wallenfeldt, K., Hulthe, J., Bokemark, L., Wikstrand, J., & Fagerberg, B. (2001). Carotid and femoral atherosclerosis, cardiovascular risk factors and C- reactive protein in relation to smokeless tobacco use or smoking in 58- year-old men. *Journal of Internal Medicine*, *250*, 492–501.

Walsh, R. A. (1994). Effects of maternal smoking on adverse pregnancy outcomes: Examination of the criteria of causation. *Human Biology*, *66*, 1059–1092.

Wang, X., Raveendran, M., & Wang, J. (2003). Genetic influence on cigarette-induced cardiovascular disease. *Progress in Cardiovascular Disease*, *45*, 361–382.

Wang, X., Tager, I. B., Van Vunakis, H., Speizer, F. E., & Hanrahan, J. P. (1997). Maternal smoking during pregnancy, urine cotinine concentrations, and birth outcomes. A prospective cohort study. *International Journal of Epidemiology*, *26*, 978–988.

Wang, X., Zuckerman, B., Pearson, C., Kaufman, G., Chen, C., Wang, G., Niu, T., Wise, P. H., Bauchner, H., & Xu, X. (2002). Maternal cigarette smoking, metabolic gene polymorphism, and infant birth weight. *The Journal of the American Medical Association*, *287*, 195–202.

Wideroe, M., Vik, T., Jacobsen, G., & Bakketeig, L. S. (2003). Does maternal smoking during pregnancy cause childhood overweight? *Paediatric and Perinatal Epidemiology*, *17*, 171–179.

Wisborg, K., Henricksen, T., Jespersen, L., & Secher, N. (2000). Nicotine patches for pregnant smokers: A randomized controlled study. *Obstetrics and Gynecology*, *96*, 967–971.

Yamamoto, T., Takanashi, S., Hasegawa, Y., Kanehira, Y., Kaizuka, M., & Okumura, K. (2003). Eotaxin level in induced sputum is increased in patients with bronchial asthma and in smokers. *Respiration*, *70*, 600–605.

Zavaroni, I., Bonini, L., Gasparini, P., Dall'Aglio, E., Passeri, M., & Reaven, G. M. (1994). Cigarette smokers are relatively glucose intolerant, hyperinsulinemic and dyslipidemic. *American Journal of Cardiology*, *73*, 904–905.

Zhang, S., Day, I., & Ye, S. (2001). Nicotine induced changes in gene expression by human coronary artery endothelial cells. *Atherosclerosis*, *154*, 277–283.