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Changes in levels of biomarkers of exposure and biological effect in a controlled study of smokers switched from conventional cigarettes to reduced-toxicant-prototype cigarettes

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ABSTRACT

Background: Development of cigarettes that reduce exposure to harmful smoke constituents is a suggested tobacco harm reduction strategy, but robust methods for measurement of change are required. We investigated whether changes in biomarkers of exposure (BoE), effective dose (BoED) and biological effect (BoBE) could be detected after switching from conventional cigarettes to a reduced-toxicant-prototype cigarette (RTP).

Methods: Regular smokers of 6–8 mg ISO tar yield cigarettes were recruited in Hamburg, Germany, and supplied with a conventional 7 mg ISO tar yield cigarette for 2 weeks then switched to the same cigarette with a different tipping paper (control) or the RTP for 6 months. Subjects smoked mostly at home and attended five residential clinic visits where urine and blood samples were collected for analysis. Primary endpoints were changes in specific biomarker levels compared with non-smoker background levels. Changes in daily cigarette consumption were also investigated.

Results: BoE levels in controls generally increased over the study period, whereas most BoE and all BoED significantly declined in RTP smokers. Most BoBE data were similar across groups and/or too variable within individuals to detect changes. Increased daily cigarette consumption was affected by supply of free cigarettes, perceived shorter smoking time per cigarette than usual brands, and perceived reduced harm.

Conclusions: Despite increased cigarette consumption, reductions in BoE and BoED were detectable.

1. Background

The health risks associated with cigarette smoking are correlated with duration of smoking and amount of daily consumption, and cessation reduces an individual's relative risks of tobacco related disease (International Agency for Research on Cancer, 2007; Doll et al., 1994). Thus, tobacco-related health risks are assumed to be due to repeated and sustained exposure to a range

Abbreviations: BoE, biomarker of exposure; BoBE, biomarker of biological effect; BoED, biomarker of effective dose; FDA, Food and Drug Administration; IOM, Institute of Medicine; ISO, International Organisation for Standardisation; ISRCTN, International Standard Randomised Controlled Trial Number; MLE, mouth level exposure; MRTP, modified risk tobacco product; PREP, potential reduced exposure prototype; RTP, reduced toxicant prototype; WHO, World Health Organisation; CPD, cigarettes per day; EOS, end of study; MFCV, modified flue-cured Virginia; CA, cellulose acetate filter material; AFR, amine-functionalised resin; HAC, high activity carbon; CU, CORESTA units; LIP, low ignition propensity; mmWG, mm water gauge; NFDPM, nicotine free dry particulate matter ('tar'); NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, N-nitrosonornicotine; NAB, N-nitrosoanabasine; NAT, N-nitrosoanatabine; PAH, polycyclic aromatic hydrocarbons; BLQ, below quantitation limit; BDL, below detection limit; HMPMA, 3-hydroxy-1-methylpropylmercapturic acid; 3-HPMA, 3-hydroxypropylmercapturic acid; MHBMA, monohydroxybutenyl mercapturic acid; CEMA, 2-cyanoethylmercapturic acid; s-ICAM-1, soluble intercellular adhesion molecule; 8-OHdG, 8-hydroxydeoxyguanosine; SOD, superoxide dismutase; GPx, glutathione peroxidase; hsCRP, high sensitivity C-reactive protein; LDL, low density lipoprotein; HDL, high density lipoprotein; MCP-1, monocyte chemotactic protein 1; LTB4, leukotriene B4; oxLDL, oxidised LDL.

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of smoke toxicants (Stratton et al., 2001). Reduction of the negative health effects of tobacco use is a clear public-health priority and has led to a series of regulatory and educational initiatives to persuade people not to smoke (World Health Organization, 2011). Despite these efforts, smoking rates in adult populations worldwide remain at 15–25%. Although numbers are declining slowly in many countries (World Health Organization, 2011), the World Health Organization (WHO) has forecast that there will be around 1.5 billion tobacco smokers worldwide in 2050 (World Health Organization, 2002). Current scientific study and public-policy debate, therefore, are concerned with whether public-health gains could arise from reducing exposure to toxicants in people who continue to use tobacco through the development of new tobacco and nicotine products.

In the 2001 Institute of Medicine (IoM) report, *Clearing the Smoke: the scientific basis for tobacco harm reduction* (Stratton et al., 2001), the development of potential reduced-exposure products (PREPs) was suggested as a possible way to achieve tobacco harm reduction. PREPs were defined as products that result in substantial reductions in exposure to one or more tobacco toxicants and that can reasonably be expected to reduce the risk of developing one or more specific diseases or adverse health effects as compared with risks conferred by use of traditional tobacco products. The IoM Report describes the types of scientific studies that might be useful for assessing potential risk reduction offered by the PREPs, including clinical studies, but the optimum study designs are still being considered (Hatsukami et al., 2009). Various researchers have since been trying to develop a validated framework for this research (Hatsukami et al., 2006).

The IoM noted the need to have initiatives related to PREPS overseen by regulators. In the USA, the Food and Drug Administration (FDA) was mandated by law to begin regulating tobacco products in 2009. The legislation includes the possibility of classifying new tobacco products as modified-risk tobacco products (MRTPs) with allowable public claims of reduced risk or exposure to toxicants as compared with traditional tobacco products. The FDA has set out draft guidance (Modified Risk Tobacco Products Applications (Draft Guidance for Industry), 2012) on the science needed to assess MRTPs, which is based partly on further findings from the IoM (Committee on Scientific Standards for Studies on Modified Risk Tobacco Products, 2011), but the optimum methods for determining products' potential to reduce tobacco-related harm remain under development.

Tobacco smoke contains a large number of toxicant species. Whether or not conventional cigarettes can be modified sufficiently to be classified as MRTPs remains unclear. Standardised machine tests, followed by various analytical methods, can collect and measure the levels of these constituents in tobacco smoke under laboratory conditions (Rickert et al., 1986). Nevertheless, cigarettes with relatively low ISO machine-measured tar yields are generally not associated with commensurately reduced health risks, partly because of compensatory smoking behaviour (11–13). Hence, beside *in vitro* and possibly *in vivo* data, assessment of MRTPs requires generating clinical data to determine whether machine-measured reductions in toxic emissions translate to reductions in human toxicant uptake and concomitant reduction of health risks in relation to comparator traditional tobacco products.

It is not entirely clear which toxicants are the most relevant to the development of smoking-associated diseases, whether there is a dose–response relationship between these toxicants and disease or how toxicants may interact in various disease pathways (Fowles and Dybing, 2003). Considerable research is being conducted, particularly with computational toxicology, to refine understanding of priority toxicants (Cunningham et al., 2011). Regulators and interested public health authorities are considering whether reductions in levels of specific cigarette smoke toxicants might yield public-health benefits. The WHO's Study Group on Tobacco Product Regulation has recommended the measurement of 18 toxicants in cigarette smoke and suggested that regulators consider setting progressively lower limits for nine of these (Burns et al., 2008). The FDA's Tobacco Products Scientific Advisory Committee has identified 93 harmful and potentially harmful constituents present in tobacco and tobacco smoke (FDA, 2012). The FDA now requires US tobacco product manufacturers to measure and disclose levels in their products of 20 of these 93 constituents, and has noted that it might in the future propose product standards that include limits on smoke toxicants (FDA, 2012).

The metabolic half-lives of many tobacco-smoke toxicants are short and changes in biomarkers of exposure (BoE) therefore, can be assessed in studies of only a few weeks' duration. We previously reported a 6-week clinical study, in which smokers switched after 2 weeks from conventional cigarettes to another conventional cigarette or to a reduced-toxicant-prototype cigarette (RTP) Shepperd et al., 2013a. The RTP had significantly reduced machine-measured yields of specific smoke toxicants. In the 4 weeks after switching, exposure to toxicants assessed by measurement of urinary BoE was also reduced. Generally, reductions in BoE reflected the machine-measured reductions of toxicants in smoke, and for some toxicants machine-measured and BoE levels reached reductions of more than 70% compared with the conventional cigarette. Sensorially, however, the RTPs were generally less acceptable to smokers than were the conventional cigarettes.

Hatsukami et al. (2006) concluded that no existing biomarkers in smokers were predictive of smoking-related disease. In the absence of such biomarkers, these authors concluded that the best way to evaluate PREPs (and presumably MRTPs) would be to assess reductions in exposure, whilst noting the distinctions between exposure reduction, risk reduction and harm reduction. Thus, a logical approach to assess relations between exposure and risk or harm is to measure biological effects. Biomarkers of biological effect (BoBE) indicate the body's response to exposure to toxicants and indicate early sub-clinical changes that might contribute to subsequent development of disease. We hypothesised that if reductions in exposure to toxicants are maintained over the longer term, BoBE levels will also be reduced by a detectable degree. We therefore developed a new RTP that aimed to reduce levels of specific toxicants whilst maintaining tar and nicotine yields and an acceptable sensory performance and performed this 6-month study to investigate whether the new RTP would alter both exposure and response to toxicants through measurement of potential BoE and BoBE. In addition to measurement of these biomarkers, endpoints included cigarette consumption and sensory impressions of the products. Longitudinal studies of tobacco consumption raise methodological and ethical issues, including accuracy of self-reporting consumption, changes in consumption because of study participation and the maintenance of smoking that may lead to an increase in lifetime risk of smoking-related diseases. For example, in our 6-week study some subjects notably increased the number of cigarettes smoked on the last day compared with average daily consumption throughout the rest of the study, which we believe was associated with their knowledge that provision of free study product was coming to an end (Shepperd et al., 2013a). Thus, in this study we obtained ethics committee approval, allowed subjects the opportunity to quit at any time during the study, and provided cessation advice throughout and after the study.

The primary objective of this study was to assess changes in selected BoE and BoBE within participants and within and between smoking groups after a forced switch from a commercial control cigarette to an RTP cigarette of equivalent International Organization for Standardization (ISO) tar yield. Secondary